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(54) ANTI-STREPTOCOCCUS SOBRINUS MONOCLONAL ANTIBODY AND CELL STRAIN FOR PRODUCING THE SAME OR KIT FOR DETECTING STREPTOCOCCUS SOBRINUS CONTAINING THE SAME

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a means for accurately and simply determining Streptococcus sobrinus.
SOLUTION: The cell strain produces a Streptococcus sobrinus specific monoclonal antibody and is obtained by a method comprising a process for subjecting a mammalian spleen cell immunized against Streptococcus sobrinus and a mammalian myeloma-derived cell to cell fusion to obtain a hybridoma and a process which is a process for cloning a cell strain capable of producing a Streptococcus sobrinus specific monoclonal antibody from the hybridoma and which assays the antibodies produced from the hybridoma with respect to combining ability with Streptococcus sobrinus and other bacteria of the genus Streptococcus.

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CLAIMS

[Claim(s)]
[Claim 1] The cell strain which is the process which is the cell strain which produces a specific monoclonal antibody to SOBURINUSU, carries out the cell fusion of the spleen cell of the mammalian which carried out mmunity by SOBURINUSU, and the cell of the myeloma origin of mammalian, and carries out cloning of process; which obtains syncytium, and the cell strain which produces a specific monoclonal antibody to SOBURINUSU out of this syncytium, and is obtained by the approach of including the process which authorizes with immunoassay the antibody produced from this syncytium about the binding affinity to SOBURINUSU and other SUTOREPUTOKKOKASU group bacteria.
[Claim 2] The National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology trust number FERM BP-7202 No. or FERM Monoclonal antibody production cell strain according to claim 1 which is BP-7203 No.
[Claim 3] The cell of the myeloma origin of said mammalian is mouse myeloma origin P3X63. Cell strain according to claim 1 which is the cell of 8.653 shares of Ag.
[Claim 4] The cell strain according to claim 1 said whose immunoassay is enzyme immunoassay (the ELISA method).
[Claim 5] The cell strain according to claim 1 said whose mammalian which carried out immunity is a mouse or a rat.
[Claim 6] The cell strain according to claim 1 said whose mammalian which carried out immunity is a BALB/C network mouse.
[Claim 7] The monoclonal antibody which is an anti-SOBURINUSU monoclonal antibody produced by the cell strain according to claim 1, has a high binding affinity in SOBURINUSU, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria.
[Claim 8] The kit which is the labelled antibody which this 1st antibody is a monoclonal antibody according to claim 7, and this 2nd antibody combines with SOBURINUSU specifically including the 1st antibody which is a kit for detecting SOBURINUSU and was combined with solid phase, and the 2nd antibody.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a monoclonal antibody specific to a SOBURINUSU bacillus (it is only described as SOBURINUSU Streptococcus sobrinus and the following), the cell strain which produces this monoclonal antibody, and the kit containing this monoclonal antibody.
[0002]
[Description of the Prior Art] SOBURINUSU is bacteria which live in the oral cavity of an animal and cause a cavity with a mu factor wardrobe bacillus (it is only described as a mu factor wardrobe Streptococcus mutans and the following). Therefore, inspection of SOBURINUSU is very useful from a viewpoint of prevention of tooth decay. For example, if a specific antibody is obtained to SOBURINUSU, prevention-of-tooth-decay inspection, the symptoms screening by the quantum of the amount of SOBURINUSU, the check of a curative effect, a prognostic judgment, etc. can be performed. However, the specific antibody was not conventionally obtained to SOBURINUSU, and the inspection approach of identifying and detecting SOBURINUSU which exists in the bacteria ensemble of the mu factor wardrobe group who was generally grown by there being no method of detecting SOBURINUSU specifically using cultivation with other mu factor wardrobe groups' bacteria (S. mutans, S.cricetus, S.rattus, etc.), and grew was enforced.
[0003] However, for detection of SOBURINUSU using cultivation, time amount 24 hours or more is needed, actuation is complicated and the quantum of SOBURINUSU is not easy actuation, either. Therefore, to perform the quantum of SOBURINUSU more correctly and simple has been desired.
[0004]
[Problem(s) to be Solved by the Invention] This invention aims at offering a means to perform the quantum of SOBURINUSU correctly and simple. This invention provides a SOBURINUSU bacillus with a specific monoclonal antibody, and offers the assay of simple SOBURINUSU by the immunity chromatography of the sandwiches method using this.
[0005] SOBURINUSU is bacteria and comparatively easy to obtain the pair of an antibody combinable in the shape of sandwiches through SOBURINUSU. However, when using a monoclonal antibody, since the antibody is uniform, the cross reaction nature of this monoclonal antibody is very important. In the monoclonal antibody to SOBURINUSU, the antibody which has a high binding affinity also not only to SOBURINUSU but to other SUTOREPUTOKKOKASU group bacteria may exist. When such an antibody is used for a sandwich technique, there is a possibility of causing trouble to the exact quantum of SOBURINUSU. Therefore, the cross reaction nature to other SUTOREPUTOKKOKASU group bacteria needs to use a low anti-SOBURINUSU monoclonal antibody.
[0006] Although this invention means solution of the above-mentioned trouble and has a high binding affinity to SOBURINUSU, it aims at offering the kit for SOBURINUSU detection with which the cross reaction nature to other SUTOREPUTOKKOKASU group bacteria contains a low antibody, the cell strain to which this is produced, and this antibody.
[0007]
[Means for Solving the Problem] This invention relates to the cell strain which produces a specific monoclonal antibody to SOBURINUSU. This cell strain The cell fusion of the spleen cell of the mammalian which carried out immunity by SOBURINUSU, and the cell of the myeloma origin of mammalian is carried out. It is the process which carries out cloning of the cell strain which produces a specific monoclonal antibody to process; and SOBURINUSU which obtain syncytium out of this syncytium. It is obtained by the approach of including the process which

authorizes with immunoassay the antibody produced from the above-mentioned syncytium about the binding affinity to SOBURINUSU and other SUTOREPUTOKKOKASU group bacteria.
[0008] Preferably, a monoclonal antibody production cell strain is the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology trust number FERM. BP-7202 No. or FERM It is BP-7203 No.
[0009] Preferably, the cell of the myeloma origin of the above-mentioned mammalian is mouse myeloma origin P3X63. It is the cell of 8.653 shares of Ag. Preferably, the above-mentioned immunoassay is enzyme immunoassay (the ELISA method).
[0010] Preferably, the mammalian which carried out [above-mentioned] immunity is a mouse or a rat.
[0011] Preferably, the mammalian which carried out [above-mentioned] immunity is a BALB/C network mouse.
[0012] This invention is one aspect of affairs, and about the anti-SOBURINUSU monoclonal antibody produced by the above-mentioned cell strain, this monoclonal antibody has a high binding affinity in SOBURINUSU, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria.
[0013] This 1st antibody is the above-mentioned monoclonal antibody including the 1st antibody by which this invention is one aspect of affairs, and this kit was combined with solid phase about the kit for detecting SOBURINUSU, and the 2nd antibody, and the 2nd antibody of the above is an antibody which is specifically combined with SOBURINUSU and by which the indicator was carried out.
[0014] The 2nd antibody of the above may be an anti-SOBURINUSU monoclonal antibody produced by the above-mentioned cell strain, or may be other antibodies which have a high binding affinity to SOBURINUSU.
[0015]
[Embodiment of the Invention] Hereafter, this invention is explained more concretely.
[0016] In this invention, especially, as long as there are no directions, well-known proteinic separation, an analysis method, and the immunological technique may be adopted in the field concerned. Such technique can be performed using a commercial enzyme, a kit, an antibody, a marker, etc.
[0017] Production of the anti-SOBURINUSU monoclonal antibody production cell strain of this invention is explained along with a production procedure below.
[0018] (Immunity) An antibody forming cell is first prepared in an animal body by carrying out immunity of the mammalian by SOBURINUSU.
[0019] As an example of "mammalian", a mouse, a rat, a cow, a rabbit, a goat, a sheep, and a guinea pig are mentioned. Mammalians are a mouse and a rat preferably and are mice more preferably. As an example of a mouse, the mouse of an A/J network, a BALB/C network, DBA / two lines, C57BL / six lines, a C3 H/helium network, a SJL network, a NZB network, and a CBA/JNCrj network is mentioned. Since the mouse of a BALB/C network shows antibody titer high in a blood serum after immunity, it can obtain a monoclonal antibody with very high compatibility with SOBURINUSU. That it is related to the ease of the ability to do of a specific hybridoma has a well-known circulating antibody potency. Moreover, generally in antibody extensive production by the ascites after establishment of a cell strain, a BALB/C network mouse is often used. By the above, the mouse of a BALB/C network is an example desirable to the immunity of SOBURINUSU. Although especially the age of a laboratory animal is not limited, it is about 4 weeks old - about 12 weeks old typically, and is about 7-weeks old mouse or a rat more preferably about 6 - 10 weeks old of abbreviation.
[0020] SOBURINUSU used for immunity may be isolated from the inside of people's oral cavity, and a commercial thing may be used for it. for example, an American type culture collection, American Maryland 20852, Rockville, Park loan drive it may receive from 12301) the law out of people's oral cavity -- according to a method, it may be isolated as a colony of a mucoid mold on MS agar medium.
[0021] SOBURINUSU may be mixed with an adjuvant before in order to reinforce an immune response before immunity. As an example of an adjuvant, a water-in-oil emulsion (for example, incomplete Freund's adjuvant), A water middle oil Nakamizu mold emulsion, an oil-in-water emulsion, liposome, aluminium hydroxide gel, Besides a silica adjuvant, a powder bentonite, and a tapioca adjuvant BCG, Propionibacterium Fungus bodies, such as acnes, fungus body component [, such as a cell wall and a trehalose DAIKO rate (TDM),]; -- the lipopolysaccharide (LPS) which is the endotoxin of a gram negative, and lipid A fraction;beta glucan (polysaccharide); -- muramyl dipeptide MDP); -- bestatin; -- synthetic compound [, such as levamisole,]; -- thymus hormone -- The protein of the biogenic substance origins, such as a thymus hormone humoral factor and a tuftsin, or peptide nature matter;, those mixture (for example, complete Freund's adjuvant), etc. are mentioned.
[0022] These adjuvants show effectiveness to enhancement or control of an immune response depending on a

route of administration, a dose, an administration stage, etc. Furthermore according to the class of adjuvant, a difference is accepted in the circulating antibody production to an antigen, induction of cell-mediated immunity, the class of an immunoglobulin, etc. So, it is desirable to choose an adjuvant appropriately according to the target immune response. The handling of the selected adjuvant, for example, the mixed approach with SOBURINUSU etc., is well-known in the field concerned.

[0023] Immunity of mammalian is performed according to a well-known approach in the field concerned. For example, a vein or intraperitoneal may be injected with SOBURINUSU which is an antigen in hypodermically [of mammalian], and a hide. Since an immune response changes with the classes and networks of mammalian by which immunity is carried out, an immunity schedule may be appropriately changed according to the animal used. An antigen challenge is repeated several times after the first immunity. A booster may be performed four weeks, six weeks, and half a year after the first immunity.

[0024] (Check of an antibody production) It checks after immunity that the antibody to SOBURINUSU is produced in the body of mammalian, and that the class switching from IgM to IgG has happened in the immunity last stage by carrying out assay of the blood which collected blood and was obtained from mammalian about existence of SOBURINUSU avidity (for example, refer to Harlow and Lane, ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, and New York (1988)). As an example of the suitable assay approach, enzyme immunoassay (the ELISA method), a radiation immunoassay method (RIA), and a fluorescent antibody technique are mentioned. It is desirable to obtain the anti-SOBURINUSU monoclonal antibody which has high compatibility to SOBURINUSU in this invention. In order to obtain the monoclonal antibody production cell of high compatibility, high antibody titer needs to be shown at the time of antiserum.

[0025] (Boost) After checking production of a SOBURINUSU affinity antibody, in order to hypertrophy a spleen, it can boost (booster injection of immunogen). Although the amount of the amount of SOBURINUSU prescribed for the patient with a boost about 4 to 5 times the amount of SOBURINUSU by which immunity is carried out first is desirable, it is not limited to this.

[0026] Typically, a boost is performed using the emulsion of SOBURINUSU and an incomplete Freund's adjuvant. However, it is desirable not to add an adjuvant but to use a pure article as SOBURINUSU prescribed for the patient for the last immunity (booster injection of the immunogen several days before cell fusion). A route of administration is suitably determined by a vein or administration intraperitoneal [each] in hypodermically and a hide in consideration of the antibody which recognizes the part where SOBURINUSU differed being obtained.

[0027] (Cell fusion) A spleen cell is extracted after the last immunity from the mammalian which carried out immunity, and cell fusion is carried out to the cell of the cell strain of the myeloma origin.

[0028] Since it is dependent on the class of cell strain of the myeloma origin used at the time of cell fusion, as for the proliferation potential force of syncytium, it is desirable to use for cell fusion the cell strain which was excellent in the proliferation potential force. Moreover, as for the cell strain of the myeloma origin, it is desirable that it is conformable with the mammalian in which the spleen cell to unite originates. The cell strain of the myeloma origin may newly be prepared and a commercial thing may be used for it. As a cell strain of the myeloma origin of a mouse, they are P3X63Ag8.653 and Sp2/O. Ag14, FO, 1, S194/5.XX0 BU.I, P3/NS 1/1 Ag4 1 etc. is mentioned. Since it becomes that in which did not produce the fragment of an antibody and the proliferation potential force of syncytium was excellent, it is P3X63. Use of Ag8.653 is desirable. As a cell strain of the rat myeloma origin, 210, RCY3.Ag.1.2.3, YB 2/0, etc. are mentioned.

[0029] Cell fusion is performed according to a well-known approach in the field concerned (refer to Koehler and Milstein, Nature 256:495[1975], Kosbor et al., 1983, Immunol. Today 4:72, Cote et al., 1983, Proc.Natl.Acad.Sci.USA, 30:2026, Cole et al., MONOCLONAL ANTIBODIES AND CANCERTHERAPY, Alan R Liss Inc., New York, NY, 77 - 96 etc. pages [1985], etc.). The approach using for example, the polyethylene-glycol method and Sendai Virus as an example of a cell fusion method, the method of using a current, etc. are mentioned. There is also comparatively little cytotoxic effect, fusion actuation is also easy, and since repeatability is high, the polyethylene-glycol method is desirable.

[0030] The obtained syncytium may be proliferated according to well-known conditions in the field concerned. Desired syncytium can be chosen based on the binding affinity of the antibody produced.

[0031] (Cell sorting and cloning) Based on a well-known approach, assay of the binding affinity of the antibody produced from syncytium may be carried out in the field concerned. In this invention, in order to obtain the syncytium which produces the antibody which has a high binding affinity in SOBURINUSU, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria, or has a low binding affinity, cloning of the target

cell strain is carried out using sorting based on the binding affinity to other SUTOREPUTOKKOKASU group bacteria. Therefore, the antibody which has a binding affinity with specific monoclonal antibody" high to SOBURINUSU to vocabulary "SOBURINUSU in this specification, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria, or has a low binding affinity is meant. The antibody has a high binding affinity in SOBURINUSU and "does not have a binding affinity" to other SUTOREPUTOKKOKASU group bacteria does not have cross reaction nature.

[0032] As for the vocabulary "it has a high binding affinity", in measurement on the same conditions, an inhibition starts substantially with the inhibition ELISA method indicated by the following example, and it means that the mesial magnitude of an inhibition is less than [abbreviation 10-6M]. Although an inhibition starts in the same measurement, it is called vocabulary "it has a low binding affinity" that the mesial magnitude of an inhibition is more than abbreviation 10-5M (for example, 10-4M, 10-3M, etc.). It is called vocabulary "it does not have a binding affinity" that an inhibition does not start in the same measurement. The vocabulary "an inhibition starts" means that the amount of the antibody combined with SOBURINUSU fixed to solid phase decreases under existence of the contention matter (inhibitor) as compared with the bottom of un-existing [of inhibitor]. It is called vocabulary "an inhibition does not start" that the amount of the antibody combined with SOBURINUSU fixed to solid phase is substantially equivalent under existence of inhibitor and un-existing. "Mesial magnitude of an inhibition" means the concentration of the inhibitor by which the absorbance of the one half of the absorbance (the amount of antibody association is reflected) under un-existing [of inhibitor] is measured.

[0033] Assay of the binding affinity of an antibody is carried out the same with having mentioned above about the check of an antibody production using approaches, such as the ELISA method, the RIA method, and a fluorescent antibody technique. Since an antibody can be detected with sufficient sensibility simple, the ELISA method is desirable.

[0034] A well-known approach may be used for cloning of syncytium in the field concerned. Limiting dilution, a soft-agar method, etc. are mentioned as an example of the approach of cloning. Actuation is also easy, and there are many track records, and since repeatability is high, limiting dilution is desirable.

[0035] In order to choose an efficient useful cell out of much syncytium obtained by cell fusion, as for cell sorting, t is desirable to carry out from the phase in early stages of cloning.

[0036] Thus, finally the syncytium stock which produces the antibody which has a desirable binding affinity is sorted out. The cell strain sorted out may be semipermanently saved in liquid nitrogen.

[0037] (Purification of an antibody) By carrying out mass culture of the monoclonal antibody production cell strain sorted out as mentioned above, a specific monoclonal antibody can be produced in large quantities to SOBURINUSU. As the mass culture approach of a monoclonal antibody production cell strain, in vivo one and in vitro culture is mentioned. As an example of in vivo mass culture, intraperitoneal [of mammalian] is injected with syncytium, and is proliferated, and the method of making antinode underwater produce an antibody is mentioned. In n vitro culture, syncytium is cultivated in a culture medium and an antibody is produced in a culture medium.

[0038] From the ascites obtained by mass culture, or a culture supernatant, the monoclonal antibody of this nvention can be refined using a well-known approach in the field concerned. a purification sake -- for example, a part for a DEAE anion-exchange chromatography, affinity chromatography, ammonium sulfate fractionation, and PEG -- a part for a drawing technique and ethanol -- a drawing technique etc. combines suitably and is used. the antibody of this invention -- usually -- about 90% of purity -- desirable -- about 95% of purity -- it is refined so that it may become about 98% of purity more preferably.

[0039] (Evaluation of an antibody) By evaluating the binding affinity of the refined monoclonal antibody, the combination of the antibody which recognizes the epitope from which it differs on a SOBURINUSU cell can be chosen from some obtained antibodies. The combination of two sorts of antibodies of arbitration which recognize a different epitope is useful because of a sandwich technique. On the other hand, since the same protein exists mostly on a bacteria front face, it is also possible to carry out a sandwich technique using one kind of monoclonal antibody.

[0040] The kit for detecting SOBURINUSU is offered in this invention. The kit of this invention may be offered in order to carry out the immunity chromatography which detects the antigen in an aquosity sample for example, based on an antigen antibody ligation reaction. The kit of this invention contains the 2nd antibody included and used for the 1st antibody and mobile phase which were combined with solid phase.

[0041] As the 1st antibody combined with solid phase, it is the trust number FERM. BP-7202 No. or trust number FERM The monoclonal antibody produced by the BP-7203 No. cell strain may be used. Suitably, it is the trust

number FERM. The monoclonal antibody produced by the BP-7202 No. cell strain may be used as the 1st antibody.

[0042] As long as it has a high binding affinity in SOBURINUSU as the 2nd antibody, the antibody of arbitration can be used, and this may be a polyclonal antibody and may be a monoclonal antibody. A monoclonal antibody is used preferably. It is the trust number FERM as the 2nd antibody. BP-7202 No. or trust number FERM The monoclonal antibody produced by the BP-7203 No. cell strain is used suitably. In this case, trust number FERM BP-7202 No. and trust number FERM Sandwiches assay can be carried out by it, using the monoclonal antibody produced by the BP-7203 No. cell strain as a pair of the lot of the 1st antibody and the 2nd antibody.

[0043] The indicator of the 2nd antibody may be carried out by the indicator of arbitration by the well-known approach in the field concerned. As an example of an indicator, enzyme labeling, a coloring matter indicator, a magnetic indicator, a radioactive indicator, the indicator by the particles (gold colloid, latex, etc.) to which the color was attached, etc. are mentioned.

[0044] The kit of this invention may be appropriately produced by the well-known approach in the field concerned. The kit of this invention may contain the 1st antibody of the above, and the 2nd antibody of the above in one or the container beyond it. A kit may contain the explanation teaching materials in the sandwiches assay of SOBURINUSU which teach use of an antibody again. A kit may contain the suitable reagent for detecting the electropositive control for detection of an indicator, and electronegative control, a washing solution, the dilution buffer solution, etc.

[0045] As mentioned above, the 1st antibody is usually fixed by solid phase, and the indicator of the 2nd antibody is carried out. The 2nd antibody is made to react with SOBURINUSU by the liquid phase, and indicator-antibody-SOBURINUSU complex is made to form first in measurement of SOBURINUSU. And the reaction mixture containing this complex is made to react with the 1st antibody solidified as a mobile phase. Consequently, the 1st antibody and 2nd antibody are combined in the shape of sandwiches through SOBURINUSU. Therefore, only when SOBURINUSU exists, an indicator is fixed on solid phase through SOBURINUSU.

[0046] Trust number FERM The anti-SOBURINUSU monoclonal antibody produced by the BP-7202 No. cell strain has a high binding affinity in SOBURINUSU, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria. Trust number FERM Similarly, the anti-SOBURINUSU monoclonal antibody produced by the BP-7203 No. cell strain also has a high binding affinity in SOBURINUSU, and does not have a binding affinity as other SUTOREPUTOKKOKASU specieses.

[0047] The fragment of the functionality holding the joint property is also contained in the "anti-SOBURINUSU monoclonal antibody" by this invention. These fragments can compete about the specific binding to the intact antibody and intact SOBURINUSU in which they originate, and can be combined with the compatibility of at least 107, 108, 109M-1, or 1010M-1. The fragment of an antibody may contain the heavy chain of an immunoglobulin, a light chain, Fab, Fab', 2 (ab'), Fabc, and Fv. The fragment of an antibody may be produced according to separation of an intact immunoglobulin chemical [like an enzyme]. For example, F(ab')2 fragment is Harlow and Lane, and ANTIBODIES:A. LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, New It can obtain from an IgG molecule using a standard approach which was indicated by York (1988) by carrying out protein digestion by the pepsin in pH 3.0-3.5. A Fab fragment can be obtained from all antibodies by restrictive reduction by the bottom papain digestion of existence of F(ab')2 fragment to a reducing agent (refer to the volume Paul and on W., 2nd edition RavanPress of FUNDAMENTAL IMMUNOLOGY, N.Y., 1989, and Chapter 7).

[0048]

[Example] Hereafter, it explains still more concretely about production of the monoclonal antibody production cell strain of this invention. This invention is not limited by the following examples.

[0049] In the example below <enzyme immunoassay (the ELISA method)>, evaluation of the obtained antiserum, a culture supernatant, and a monoclonal antibody was performed by the ELISA method. The operation information is indicated below.

[0050] (A) Coating SOBURINUSU of an antigen (SOBURINUSU) was prepared to 108 cells / concentration of mL. 100microl / well impregnation was carried out to the microplate (high [made from polystyrene] joint mold flat bottom # 2580, coaster company make), and the antigen solution was saved in saturated steam at the room temperature overnight at it. The aspirator removed the antigen solution just before the experiment.

[0051] (B) 200microl / well impregnation of 1 % of the weight BSA-PBS-Az (Az: aza-id sodium salt) of blocking were carried out, and it was left at the room temperature for 30 minutes. Then, the aspirator removed 1 % of the weight BSA-PBS-Az. When not conducting subsequent experiments on the same day, it is in this condition and

saved at 4 degrees C in saturated steam.
[0052] (C) The antibody solutions (antiserum, a culture supernatant, purification antibody, etc.) diluted to concentration various by 1 % of the weight BSA-PBS-Az of reactions of an antibody were poured in by 50microl / well, and 1 % of the weight BSA-PBS-Az was poured in by 50microl / well. After half[1 hour and]-leaving it in ordinary temperature, PBS which washes 3 times by PBS and remains with an aspirator was removed.
[0053] (D) 50microl / well impregnation of the thing which dissolved the anti-mouse IgG antibody (product made from KPL) of the goat origin in which 0.2micro g/mL of reactions of the 2nd antibody carried out peroxidase labeling in the PBS solution of BSA 1% of the weight, or the thing which dissolved the anti-mouse IgM antibody (product made from KPL) of the goat origin in which 0.2microg/mL carried out peroxidase labeling in the PBS solution of BSA 1% of the weight were carried out, and it was left in ordinary temperature for 30 minutes. PBS which washes 3 times by PBS and remains with an aspirator further was removed.
[0054] (E) The reaction of a substrate and halt O-phenylenediamine (for biochemistry) 40mg were dissolved in the citric-acid-phosphoric-acid buffer (pH5) of 10mL(s), 100microl / well impregnation of the solution (substrate solution) which added 4micro of hydrogen peroxide solution L 30% of the weight just before use were carried out, and room temperature neglect was carried out. After about 3 minutes, 25microl / well impregnation of the 4-N sulfuric acid were carried out, and the reaction was suspended.
[0055] (F) The absorbance of 492nm was measured using the measurement microplate reader (Oriental soda company make).
[0056] In addition, although enzyme immunoassay was used as immunoassay in this example, the RIA method, a fluorescent antibody technique, etc. may be used for others.
[0057] In <example> this example, the BALB/C network mouse was used for immunity, taking that there is a track record at this invention persons' lab, and that a BALB/C network mouse is most often used in the antibody mass culture by the ascites after monoclonal antibody production cell strain establishment into consideration.
[0058] (Immunity) SOBURINUSU which is immunogen was prepared to 108 cells / mL using the physiology salt concentration phosphate buffer solution (PBS). The adjuvant emulsion containing immunogen was obtained by adding the adjuvant (a Homo sapiens tubercular killed bacteria content complete Freund's adjuvant, the Wako Pure Chem make, H37Rv) of this volume in the PBS solution of this SOBURINUSU, and fully emulsifying by engine-speed 1000rpm by the homogenizer in it.
[0059] 15 mice (BALB/C) of the female of about seven weeks of after the birth were injected with immunogen, and intraperitoneal or hypodermically were injected with the **** adjuvant emulsion every [100micro / l]. The incomplete Freund's adjuvant of the SOBURINUSU solution and this which were prepared to 108 cells / mL using PBS, and this volume was emulsified by the homogenizer after two weeks, and the part same to a BALB/C mouse as last time was injected with this emulsion every [100micro / l].
[0060] Then, the mouse was injected with imperfect furoin tor undergarment TOEMARUJON which contains SOBURINUSU of the same presentation as the immunity of two weeks after, and concentration after four weeks, six weeks, and half a year from immunity initiation to the 100micro part as last time same [every / l]. It collected blood the one-week back of the 2nd injection, and after [of the 5th injection] one week, respectively, and the antibody production shown below was checked.
[0061] (Check of an antibody production) The blood serum was separated from the extracted blood and the antibody production was checked with enzyme immunoassay (the ELISA method) using the obtained blood serum. The 108 cells / mL, and SOBURINUSU prepared by PBS-Az as solid phase were poured distributively 100microl / well every, and the microplate which carried out the coat at the room temperature overnight was used. The peroxidase-labeling anti-mouse IgG antibody or the peroxidase-labeling anti-mouse IgM antibody was used as the second antibody. It is checked that the antibody combined with SOBURINUSU exists in an antibody sample by coloring in a well.
[0062] Consequently, production of an anti-SOBURINUSU antibody was accepted in the mice of all 15 animals. Furthermore, also in which mouse, it was checked that the antibody production has shifted from IgG to IgM after the 2nd injection, and after injection which is the 5th time, an IgG/IgM ratio is 300 or more and it checked that the class switching had happened enough.
[0063] (Cell fusion) The last immunity was performed in order to hypertrophy the spleen of three animals which was high as for especially the potency in the mouse which carried out immunity. SOBURINUSU of immunogen was prepared to 108 cells / concentration of mL six months after immunity initiation using PBS, and the mouse was injected every [100micro / l], without adding an adjuvant.

[0064] The spleen cell of one animal was extracted among the mice which passed three days after the last immunity. Using the polyethylene glycol of a mean molecular weight 1,500, with the conventional method, the spleen cell and the mouse myeloma origin cell strain (P3X63 Ag8.653) were united, and syncytium was obtained.
[0065] It wound around one 96 well plate, after making the hypoxanthine / aminopterin / thymidine (HAT) culture medium which prepared syncytium by the ISHIKOFU culture medium containing 15% of the weight of fetal calf serum (following, FCS) float (200microl / well). Under the present circumstances, the feeder cell (cell which supplies a growth factor at the time of culture initiation) used the spleen cell of the same mouse individual. Culture was started within the CO2 incubator (CO2 concentration: five volume %, temperature:37 degree C, humidity:95%). By the following culture, unless it was shown in others, it cultivated on the same conditions as this.
[0066] (Cell sorting and cloning) one -- a week -- after -- syncytium -- a culture supernatant -- 100 -- micro -- l -- extraction -- having carried out -- after -- syncytium -- containing -- the remainder -- culture medium -- four -- a sheet -- 24 -- a well -- a plate -- a passage -- carrying out -- each -- a well -- one -- ml -- 15 -- % of the weight -- FCS -- containing -- hypoxanthine -- /-- thymidine -- (-- HT --) -- a culture medium -- having added .
[0067] Four days after carrying out the passage of the syncytium to 24 well plate, cell culture supernatant liquid was extracted 150microl / well every. The binding affinity to SOBURINUSU was measured by the ELISA method shown below using this culture supernatant and the culture supernatant extracted after culture initiation at the 1st week.
[0068] SOBURINUSU prepared to the concentration of 5microg/mL by 0.1 mg/mL-BSA-PBS-Az as solid phase was used 100microl / well every. Cell culture supernatant liquid was used as antibody liquid. The peroxidase-labeling anti-mouse IgG antibody was used as the 2nd antibody.
[0069] The ELISA method result of the culture supernatant extracted twice was doubled, and 20 good wells of a vegetative state which have a high binding affinity to SOBURINUSU were checked. As the 1st-step selection, the passage of all of the cell of these wells was carried out to four 6 well plates, and the **** HT culture medium was added for 15% of the weight of 4ml FCS to each well.
[0070] The culture supernatant was extracted two days after cell sorting of the 1st step, and the binding affinity to SOBURINUSU was measured by the ELISA method shown below.
[0071] SOBURINUSU prepared to 108 cells / concentration of mL by PBS-Az as solid phase was used 100microl / well every. Cell culture supernatant liquid was used as antibody liquid. As the second antibody, the peroxidase-labeling anti-mouse IgG antibody was used.
[0072] Consequently, 10 well sorting of the well with a high binding affinity was carried out to SOBURINUSU. The passage of the cell of these wells was carried out to the inside flask (capacity of 50ml), respectively. The culture medium added 45ml of HT culture media containing 15% of the weight of FCS at a time.
[0073] The culture supernatant was extracted passage days [three days] after the cell which received the 2nd-step selection, and the binding affinity to SOBURINUSU and other SUTOREPUTOKKOKASU kinds (streptococcus mu factor wardrobe) was measured by the ELISA method shown below.
[0074] As solid phase, the mu factor wardrobe prepared to 108 cells / concentration of mL by PBS-Az was used 100microl / well every. Cell culture supernatant liquid was used as antibody liquid. As the second antibody, the peroxidase-labeling anti-mouse IgG antibody was used. If the antibody combined with a mu factor wardrobe exists in cell culture supernatant liquid, it will color on a well.
[0075] Only SOBURINUSU showed the binding affinity and 2 well sorting of the well which does not show a binding affinity to the mu factor wardrobe which are other SUTOREPUTOKKOKASU group bacteria was carried out.
[0076] About the cell of the two above-mentioned well, using HT culture medium containing 15% of the weight of FCS, it diluted to the concentration in which two cells per one well are contained (limiting dilution), and poured distributively each to two microplates of 96 wells. Initial growth was urged using the thymocyte of the mouse (BALB/C) of the female of four weeks of after the birth as a feeder. Culture was advanced raising the size of a plate and screening by the above-mentioned ELISA method was repeated about cell culture supernatant liquid timely. Finally the cell strain in which a high potency is shown and good growth is shown to SOBURINUSU was sorted out, and culture was advanced until it resulted in 5x105 cells / concentration of mL in the culture medium of 200mL. Finally, two shares of stocks which have a high binding affinity and do not start a cross reaction to other SUTOREPUTOKKOKASU group bacteria to SOBURINUSU were selected.
[0077] One share of the inside which showed the high binding affinity to SOBURINUSU was named cell strain name:SS-1, and domestic deposition was carried out in National Institute of Bioscience and Human-Technology,

Agency of Industrial Science and Technology on June 30, Heisei 12 (trust number FERM BP No. 7202).
[0078] One more share with a high binding affinity was similarly named cell strain name:SS-2 to SOBURINUSU, and domestic deposition was carried out in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on June 30, Heisei 12 (trust number FERM BP No. 7203).
[0079] The antibody (SS-1 share and SS-2 share) to produce is called SS1 antibody and SS2 antibody, respectively.
[0080] (Preservation of a cell) After it carried out centrifugal separation of the cell strain finally sorted out, and it removed supernatant liquid, made solution 1mL of FCS:dimethyl sulfoxide =9:1 (volume ratio) float by 1x107 cells / concentration of mL and carried out preliminary freezing at -80 degrees C, it was moved into liquid nitrogen and changed into the mothball condition.
[0081] (Purification of an antibody) Mass culture of the two selected shares was carried out by the ISHIKOFU culture medium which contains FCS 15% of the weight, respectively, and centrifugal separation of the supernatant liquid was carried out. Moreover, inject intraperitoneal [of a female BALB/C mouse] with two selected shares, respectively, it was made to increase, and ascites was stored up. The accumulated ascites was extracted. The culture supernatant or ascites of each stock was poured on the affinity chromatography using protein A joint gel (protein A sepharose 4FF, Pharmacia manufacture), and each monoclonal antibody (SS1 antibody and SS2 antibody) was refined on condition that the following.
[0082] The column filled up with protein A joint gel was equilibrated with the joint buffer solution (a 1.5M glycine and 3M NaCl, pH8.9). After diluting a culture supernatant or ascites with the joint buffer solution about 3 times, it was applied to the column which equilibrated. The joint buffer solution washed the column until the elution of an impurity was completed acting as the monitor of the eluate from a column by 280nm. The elution buffer solution (a 100mM citric acid, pH4) was applied to the column after washing (linear flow rate : about 20cm/(hour)), and IgG content eluates were collected. About the collected IgG content eluate, the absorbance of 280nm was measured with the absorptiometer and the concentration of an antibody was determined by converting the measured absorbance with an absorbancy index.
[0083] From the comparison with the standard protein by SDS polyacrylamide gel electrophoresis, each purification fractionation of these monoclonal antibodies (SS1 and SS2) checked that it was IgG which consists of molecular weight about 50,000 H chain, and about 25,000 L chain. In addition, mixing of an impurity was below limit of detection on electrophoresis.
[0084] (Evaluation of an antibody) About two kinds of monoclonal antibodies refined by the above-mentioned affinity chromatography, antibody evaluation was performed using the dilution sequence of SOBURINUSU on the same conditions as the inhibition ELISA method in the 2nd-step [above-mentioned] selection.
[0085] Drawing 1 is a graph which shows the result of having measured the binding affinity [respectively as opposed to / antibody / SS1 / similarly / antibody / SS2 / (a) SOBURINUSU (white round head) and (b) mu factor wardrobe (black dot) in drawing 2].
[0086] As shown in drawing 1 , by SS1 antibody, to association to SOBURINUSU having been observed with x1 - x1/10 diluent, the mu factor wardrobe was received, and association was not observed about the diluent of a gap, either, but it was shown that SOBURINUSU can be specifically detected by SS1 antibody.
[0087] Similarly, as shown in drawing 2 , the mu factor wardrobe was received to association to SOBURINUSU having been observed with x1 - x1/100 diluent also in SS2 antibody, and association was not observed about the diluent of a gap, either, but it was shown that SOBURINUSU can be specifically detected by SS2 antibody.
[0088] (Sandwiches reaction) In the ELISA method, the coat of the SS1 antibody is carried out to a plate, SOBURINUSU was combined, after making SS2 antibody which carried out the enzyme label react, the excessive antibody was removed, and sufficient coloring was obtained, when the chromophoric substrate was added and having been incubated. That is, the combination of SS1 antibody, and an SS2 antibody or a polyclonal antibody has checked the useful thing to the inspection approach using sandwiches reactions, such as an immunity chromatography.
[0089] (Detection sensitivity in an immunity chromatography) SS2 antibody which fixed and carried out the gold colloid indicator of the SS1 antibody on the filter paper according to the conventional method was put on the mobile phase, and immunity chromatography equipment was produced. When the sample which contains SOBURINUSU by various concentration was applied, the sensibility of this immunity chromatography equipment was about 106 cells / ml.
[0090] Generally, it is known that the SOBURINUSURE bells in healthy people's inside of the oral cavity are about

104 to 107 cell / ml. Therefore, the immunity chromatography equipment produced using the antibody of this invention can detect SOBURINUSU in the inside of the oral cavity.

[0091]

[Effect of the Invention] According to the production approach of the monoclonal antibody production cell strain of this invention, in cloning of a cell strain, the binding affinity to SOBURINUSU of the antibody produced from syncytium and other SUTOREPUTOKKOKASU kinds is authorized, and the target cell is sorted out. Therefore, an efficient useful cell can be chosen from many cells which exist in early stages after cell fusion. And the cell strain which produces an antibody with high singularity can be produced, attaining the high compatibility over SOBURINUSU.

[0092] When immunoassay is enzyme immunoassay (the ELISA method), the binding affinity of an antibody can be authorized with sufficient sensibility simple.

[0093] The cell strain of the myeloma origin of mammalian is mouse myeloma origin P3X63. When it is Ag8.653, the fragment of an antibody is not produced, but since especially the proliferation potential force of the syncytium obtained further is excellent, many cells can be authorized in a short time.

[0094] When the mammalian which carries out immunity is a mouse or a rat, it is convenient in respect of the handling of an animal, and immunization. When the mammalian which carries out immunity is a BALB/C network mouse, compatibility with SOBURINUSU becomes possible [obtaining a very high monoclonal antibody].

[0095] According to the monoclonal antibody production cell strain of this invention, by cultivating it, it has the high compatibility over SOBURINUSU and an anti-SOBURINUSU monoclonal antibody with high singularity can be offered semipermanently.

[0096] If it is used for a sandwich technique combining the monoclonal antibody and anti-SOBURINUSU polyclonal antibody of this invention, combining SS1 antibody and SS2 antibody which are a monoclonal antibody of this invention, a high unique SOBURINUSU detection kit can be offered.

[Translation done.]

* NOTICES *

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2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

TECHNICAL FIELD

[Field of the Invention] This invention relates to a monoclonal antibody specific to a SOBURINUSU bacillus (it is only described as SOBURINUSU Streptococcus sobrinus and the following), the cell strain which produces this monoclonal antibody, and the kit containing this monoclonal antibody.

[Translation done.]

PRIOR ART

[Description of the Prior Art] SOBURINUSU is bacteria which live in the oral cavity of an animal and cause a cavity with a mu factor wardrobe bacillus (it is only described as a mu factor wardrobe Streptococcus mutans and the following). Therefore, inspection of SOBURINUSU is very useful from a viewpoint of prevention of tooth decay. For example, if a specific antibody is obtained to SOBURINUSU, prevention-of-tooth-decay inspection, the symptoms screening by the quantum of the amount of SOBURINUSU, the check of a curative effect, a prognostic judgment, etc. can be performed. However, the specific antibody was not conventionally obtained to SOBURINUSU, and the inspection approach of identifying and detecting SOBURINUSU which exists in the bacteria ensemble of the mu factor wardrobe group who was generally grown by there being no method of detecting SOBURINUSU specifically using cultivation with other mu factor wardrobe groups' bacteria (S. mutans, S.cricetus, S.rattus, etc.), and grew was enforced.

[0003] However, for detection of SOBURINUSU using cultivation, time amount 24 hours or more is needed, actuation is complicated and the quantum of SOBURINUSU is not easy actuation, either. Therefore, to perform the quantum of SOBURINUSU more correctly and simple has been desired.

[Translation done.]

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EFFECT OF THE INVENTION

[Effect of the Invention] According to the production approach of the monoclonal antibody production cell strain of this invention, in cloning of a cell strain, the binding affinity to SOBURINUSU of the antibody produced from syncytium and other SUTOREPUTOKKOKASU kinds is authorized, and the target cell is sorted out. Therefore, an efficient useful cell can be chosen from many cells which exist in early stages after cell fusion. And the cell strain which produces an antibody with high singularity can be produced, attaining the high compatibility over SOBURINUSU.

[0092] When immunoassay is enzyme immunoassay (the ELISA method), the binding affinity of an antibody can be authorized with sufficient sensibility simple.

[0093] The cell strain of the myeloma origin of mammalian is mouse myeloma origin P3X63. When it is Ag8.653, the fragment of an antibody is not produced, but since especially the proliferation potential force of the syncytium obtained further is excellent, many cells can be authorized in a short time.

[0094] When the mammalian which carries out immunity is a mouse or a rat, it is convenient in respect of the handling of an animal, and immunization. When the mammalian which carries out immunity is a BALB/C network mouse, compatibility with SOBURINUSU becomes possible [obtaining a very high monoclonal antibody].

[0095] According to the monoclonal antibody production cell strain of this invention, by cultivating it, it has the high compatibility over SOBURINUSU and an anti-SOBURINUSU monoclonal antibody with high singularity can be offered semipermanently.

[0096] If it is used for a sandwich technique combining the monoclonal antibody and anti-SOBURINUSU polyclonal antibody of this invention, combining SS1 antibody and SS2 antibody which are a monoclonal antibody of this invention, a high unique SOBURINUSU detection kit can be offered.

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] This invention aims at offering a means to perform the quantum of SOBURINUSU correctly and simple. This invention provides a SOBURINUSU bacillus with a specific monoclonal antibody, and offers the assay of simple SOBURINUSU by the immunity chromatography of the sandwiches method using this.

[0005] SOBURINUSU is bacteria and comparatively easy to obtain the pair of an antibody combinable in the shape of sandwiches through SOBURINUSU. However, when using a monoclonal antibody, since the antibody is uniform, the cross reaction nature of this monoclonal antibody is very important. In the monoclonal antibody to SOBURINUSU, the antibody which has a high binding affinity also not only to SOBURINUSU but to other SUTOREPUTOKKOKASU group bacteria may exist. When such an antibody is used for a sandwich technique, there is a possibility of causing trouble to the exact quantum of SOBURINUSU. Therefore, the cross reaction nature to other SUTOREPUTOKKOKASU group bacteria needs to use a low anti-SOBURINUSU monoclonal antibody.

[0006] Although this invention means solution of the above-mentioned trouble and has a high binding affinity to SOBURINUSU, it aims at offering the kit for SOBURINUSU detection with which the cross reaction nature to other SUTOREPUTOKKOKASU group bacteria contains a low antibody, the cell strain to which this is produced, and this antibody.

[Translation done.]

MEANS

[Means for Solving the Problem] This invention relates to the cell strain which produces a specific monoclonal antibody to SOBURINUSU. This cell strain The cell fusion of the spleen cell of the mammalian which carried out immunity by SOBURINUSU, and the cell of the myeloma origin of mammalian is carried out. It is the process which carries out cloning of the cell strain which produces a specific monoclonal antibody to process; and SOBURINUSU which obtain syncytium out of this syncytium. It is obtained by the approach of including the process which authorizes with immunoassay the antibody produced from the above-mentioned syncytium about the binding affinity to SOBURINUSU and other SUTOREPUTOKKOKASU group bacteria.

[0008] Preferably, a monoclonal antibody production cell strain is the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology trust number FERM. BP-7202 No. or FERM It is BP-7203 No.

[0009] Preferably, the cell of the myeloma origin of the above-mentioned mammalian is mouse myeloma origin P3X63. It is the cell of 8.653 shares of Ag. Preferably, the above-mentioned immunoassay is enzyme immunoassay (the ELISA method).

[0010] Preferably, the mammalian which carried out [above-mentioned] immunity is a mouse or a rat.

[0011] Preferably, the mammalian which carried out [above-mentioned] immunity is a BALB/C network mouse.

[0012] This invention is one aspect of affairs, and about the anti-SOBURINUSU monoclonal antibody produced by the above-mentioned cell strain, this monoclonal antibody has a high binding affinity in SOBURINUSU, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria.

[0013] This 1st antibody is the above-mentioned monoclonal antibody including the 1st antibody by which this invention is one aspect of affairs, and this kit was combined with solid phase about the kit for detecting SOBURINUSU, and the 2nd antibody, and the 2nd antibody of the above is an antibody which is specifically combined with SOBURINUSU and by which the indicator was carried out.

[0014] The 2nd antibody of the above may be an anti-SOBURINUSU monoclonal antibody produced by the above-mentioned cell strain, or may be other antibodies which have a high binding affinity to SOBURINUSU.

[0015]

[Embodiment of the Invention] Hereafter, this invention is explained more concretely.

[0016] In this invention, especially, as long as there are no directions, well-known proteinic separation, an analysis method, and the immunological technique may be adopted in the field concerned. Such technique can be performed using a commercial enzyme, a kit, an antibody, a marker, etc.

[0017] Production of the anti-SOBURINUSU monoclonal antibody production cell strain of this invention is explained along with a production procedure below.

[0018] (Immunity) An antibody forming cell is first prepared in an animal body by carrying out immunity of the mammalian by SOBURINUSU.

[0019] As an example of "mammalian", a mouse, a rat, a cow, a rabbit, a goat, a sheep, and a guinea pig are mentioned. Mammalians are a mouse and a rat preferably and are mice more preferably. As an example of a mouse, the mouse of an A/J network, a BALB/C network, DBA / two lines, C57BL / six lines, a C3 H/helium network, a SJL network, a NZB network, and a CBA/JNCrj network is mentioned. Since the mouse of a BALB/C network shows antibody titer high in a blood serum after immunity, it can obtain a monoclonal antibody with very high compatibility with SOBURINUSU. That it is related to the ease of the ability to do of a specific hybridoma has a well-known circulating antibody potency. Moreover, generally in antibody extensive production by the ascites after establishment of a cell strain, a BALB/C network mouse is often used. By the above, the mouse of a BALB/C network is an example desirable to the immunity of SOBURINUSU. Although especially the age of a laboratory

animal is not limited, it is about 4 weeks old - about 12 weeks old typically, and is about 7-weeks old mouse or a rat more preferably about 6 - 10 weeks old of abbreviation.

[0020] SOBURINUSU used for immunity may be isolated from the inside of people's oral cavity, and a commercial (for example, an American type culture collection, American Maryland 20852, Rockville, Park loan drive it may receive from 12301) thing may be used for it. the law out of people's oral cavity -- according to a method, it may be isolated as a colony of a mucoid mold on MS agar medium.

[0021] SOBURINUSU may be mixed with an adjuvant before in order to reinforce an immune response before immunity. As an example of an adjuvant, a water-in-oil emulsion (for example, incomplete Freund's adjuvant), A water middle oil Nakamizu mold emulsion, an oil-in-water emulsion, liposome, aluminium hydroxide gel, Besides a silica adjuvant, a powder bentonite, and a tapioca adjuvant BCG, Propionibacterium Fungus bodies, such as acnes, fungus body component [, such as a cell wall and a trehalose DAIKO rate (TDM),]; -- the lipopolysaccharide (LPS) which is the endotoxin of a gram negative, and lipid A fraction;beta glucan (polysaccharide); -- muramyl dipeptide (MDP); -- bestatin; -- synthetic compound [, such as levamisole,]; -- thymus hormone -- The protein of the biogenic substance origins, such as a thymus hormone humoral factor and a tuftsin, or peptide nature matter;, those mixture (for example, complete Freund's adjuvant), etc. are mentioned.

[0022] These adjuvants show effectiveness to enhancement or control of an immune response depending on a route of administration, a dose, an administration stage, etc. Furthermore according to the class of adjuvant, a difference is accepted in the circulating antibody production to an antigen, induction of cell-mediated immunity, the class of an immunoglobulin, etc. So, it is desirable to choose an adjuvant appropriately according to the target immune response. The handling of the selected adjuvant, for example, the mixed approach with SOBURINUSU etc., is well-known in the field concerned.

[0023] Immunity of mammalian is performed according to a well-known approach in the field concerned. For example, a vein or intraperitoneal may be injected with SOBURINUSU which is an antigen in hypodermically [of mammalian], and a hide. Since an immune response changes with the classes and networks of mammalian by which immunity is carried out, an immunity schedule may be appropriately changed according to the animal used. An antigen challenge is repeated several times after the first immunity. A booster may be performed four weeks, six weeks, and half a year after the first immunity.

[0024] (Check of an antibody production) It checks after immunity that the antibody to SOBURINUSU is produced in the body of mammalian, and that the class switching from IgM to IgG has happened in the immunity last stage by carrying out assay of the blood which collected blood and was obtained from mammalian about existence of SOBURINUSU avidity (for example, refer to Harlow and Lane, ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, and New York (1988)). As an example of the suitable assay approach, enzyme immunoassay (the ELISA method), a radiation immunoassay method (RIA), and a fluorescent antibody technique are mentioned. It is desirable to obtain the anti-SOBURINUSU monoclonal antibody which has high compatibility to SOBURINUSU in this invention. In order to obtain the monoclonal antibody production cell of high compatibility, high antibody titer needs to be shown at the time of antiserum.

[0025] (Boost) After checking production of a SOBURINUSU affinity antibody, in order to hypertrophy a spleen, it can boost (booster injection of immunogen). Although the amount of the amount of SOBURINUSU prescribed for the patient with a boost about 4 to 5 times the amount of SOBURINUSU by which immunity is carried out first is desirable, it is not limited to this.

[0026] Typically, a boost is performed using the emulsion of SOBURINUSU and an incomplete Freund's adjuvant. However, it is desirable not to add an adjuvant but to use a pure article as SOBURINUSU prescribed for the patient for the last immunity (booster injection of the immunogen several days before cell fusion). A route of administration is suitably determined by a vein or administration intraperitoneal [each] in hypodermically and a hide in consideration of the antibody which recognizes the part where SOBURINUSU differed being obtained.

[0027] (Cell fusion) A spleen cell is extracted after the last immunity from the mammalian which carried out immunity, and cell fusion is carried out to the cell of the cell strain of the myeloma origin.

[0028] Since it is dependent on the class of cell strain of the myeloma origin used at the time of cell fusion, as for the proliferation potential force of syncytium, it is desirable to use for cell fusion the cell strain which was excellent in the proliferation potential force. Moreover, as for the cell strain of the myeloma origin, it is desirable that it is conformable with the mammalian in which the spleen cell to unite originates. The cell strain of the myeloma origin may newly be prepared and a commercial thing may be used for it. As a cell strain of the myeloma origin of a mouse, they are P3X63Ag8.653 and Sp2/O. Ag14, FO, 1, S194/5.XX0 BU.I, P3/NS 1/1 Ag4 1 etc. is

mentioned. Since it becomes that in which did not produce the fragment of an antibody and the proliferation potential force of syncytium was excellent, it is P3X63. Use of Ag8.653 is desirable. As a cell strain of the rat myeloma origin, 210, RCY3.Ag.1.2.3, YB 2/0, etc. are mentioned.

[0029] Cell fusion is performed according to a well-known approach in the field concerned (refer to Koehler and Milstein, Nature 256:495[1975], Kosbor et al., 1983, Immunol. Today 4:72, Cote et al., 1983, Proc.Natl.Acad.Sci.USA, 80:2026, Cole et al., MONOCLONAL ANTIBODIES AND CANCERTHERAPY, Alan R Liss Inc., New York, NY, 77 - 96 etc. pages [1985], etc.). The approach using for example, the polyethylene-glycol method and Sendai Virus as an example of a cell fusion method, the method of using a current, etc. are mentioned. There is also comparatively little cytotoxic effect, fusion actuation is also easy, and since repeatability is high, the polyethylene-glycol method is desirable.

[0030] The obtained syncytium may be proliferated according to well-known conditions in the field concerned. Desired syncytium can be chosen based on the binding affinity of the antibody produced.

[0031] (Cell sorting and cloning) Based on a well-known approach, assay of the binding affinity of the antibody produced from syncytium may be carried out in the field concerned. In this invention, in order to obtain the syncytium which produces the antibody which has a high binding affinity in SOBURINUSU, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria, or has a low binding affinity, cloning of the target cell strain is carried out using sorting based on the binding affinity to other SUTOREPUTOKKOKASU group bacteria. Therefore, the antibody which has a binding affinity with specific monoclonal antibody" high to SOBURINUSU to vocabulary "SOBURINUSU in this specification, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria, or has a low binding affinity is meant. The antibody has a high binding affinity in SOBURINUSU and "does not have a binding affinity" to other SUTOREPUTOKKOKASU group bacteria does not have cross reaction nature.

[0032] As for the vocabulary "it has a high binding affinity", in measurement on the same conditions, an inhibition starts substantially with the inhibition ELISA method indicated by the following example, and it means that the mesial magnitude of an inhibition is less than [abbreviation 10-6M]. Although an inhibition starts in the same measurement, it is called vocabulary "it has a low binding affinity" that the mesial magnitude of an inhibition is more than abbreviation 10-5M (for example, 10-4M, 10-3M, etc.). It is called vocabulary "it does not have a binding affinity" that an inhibition does not start in the same measurement. The vocabulary "an inhibition starts" means that the amount of the antibody combined with SOBURINUSU fixed to solid phase decreases under existence of the contention matter (inhibitor) as compared with the bottom of un-existing [of inhibitor]. It is called vocabulary "an inhibition does not start" that the amount of the antibody combined with SOBURINUSU fixed to solid phase is substantially equivalent under existence of inhibitor and un-existing. "Mesial magnitude of an inhibition" means the concentration of the inhibitor by which the absorbance of the one half of the absorbance (the amount of antibody association is reflected) under un-existing [of inhibitor] is measured.

[0033] Assay of the binding affinity of an antibody is carried out the same with having mentioned above about the check of an antibody production using approaches, such as the ELISA method, the RIA method, and a fluorescent antibody technique. Since an antibody can be detected with sufficient sensibility simple, the ELISA method is desirable.

[0034] A well-known approach may be used for cloning of syncytium in the field concerned. Limiting dilution, a soft-agar method, etc. are mentioned as an example of the approach of cloning. Actuation is also easy, and there are many track records, and since repeatability is high, limiting dilution is desirable.

[0035] In order to choose an efficient useful cell out of much syncytium obtained by cell fusion, as for cell sorting, it is desirable to carry out from the phase in early stages of cloning.

[0036] Thus, finally the syncytium stock which produces the antibody which has a desirable binding affinity is sorted out. The cell strain sorted out may be semipermanently saved in liquid nitrogen.

[0037] (Purification of an antibody) By carrying out mass culture of the monoclonal antibody production cell strain sorted out as mentioned above, a specific monoclonal antibody can be produced in large quantities to SOBURINUSU. As the mass culture approach of a monoclonal antibody production cell strain, in vivo one and in vitro culture is mentioned. As an example of in vivo mass culture, intraperitoneal [of mammalian] is injected with syncytium, and is proliferated, and the method of making antinode underwater produce an antibody is mentioned. In in vitro culture, syncytium is cultivated in a culture medium and an antibody is produced in a culture medium.

[0038] From the ascites obtained by mass culture, or a culture supernatant, the monoclonal antibody of this invention can be refined using a well-known approach in the field concerned. a purification sake -- for example, a

part for a DEAE anion-exchange chromatography, affinity chromatography, ammonium sulfate fractionation, and PEG -- a part for a drawing technique and ethanol -- a drawing technique etc. combines suitably and is used. the antibody of this invention -- usually -- about 90% of purity -- desirable -- about 95% of purity -- it is refined so that it may become about 98% of purity more preferably.

[0039] (Evaluation of an antibody) By evaluating the binding affinity of the refined monoclonal antibody, the combination of the antibody which recognizes the epitope from which it differs on a SOBURINUSU cell can be chosen from some obtained antibodies. The combination of two sorts of antibodies of arbitration which recognize a different epitope is useful because of a sandwich technique. On the other hand, since the same protein exists mostly on a bacteria front face, it is also possible to carry out a sandwich technique using one kind of monoclonal antibody.

[0040] The kit for detecting SOBURINUSU is offered in this invention. The kit of this invention may be offered in order to carry out the immunity chromatography which detects the antigen in an aquosity sample for example, based on an antigen antibody ligation reaction. The kit of this invention contains the 2nd antibody included and used for the 1st antibody and mobile phase which were combined with solid phase.

[0041] As the 1st antibody combined with solid phase, it is the trust number FERM. BP-7202 No. or trust number FERM The monoclonal antibody produced by the BP-7203 No. cell strain may be used. Suitably, it is the trust number FERM. The monoclonal antibody produced by the BP-7202 No. cell strain may be used as the 1st antibody.

[0042] As long as it has a high binding affinity in SOBURINUSU as the 2nd antibody, the antibody of arbitration can be used, and this may be a polyclonal antibody and may be a monoclonal antibody. A monoclonal antibody is used preferably. It is the trust number FERM as the 2nd antibody. BP-7202 No. or trust number FERM The monoclonal antibody produced by the BP-7203 No. cell strain is used suitably. In this case, trust number FERM BP-7202 No. and trust number FERM Sandwiches assay can be carried out by it, using the monoclonal antibody produced by the BP-7203 No. cell strain as a pair of the lot of the 1st antibody and the 2nd antibody.

[0043] The indicator of the 2nd antibody may be carried out by the indicator of arbitration by the well-known approach in the field concerned. As an example of an indicator, enzyme labeling, a coloring matter indicator, a magnetic indicator, a radioactive indicator, the indicator by the particles (gold colloid, latex, etc.) to which the color was attached, etc. are mentioned.

[0044] The kit of this invention may be appropriately produced by the well-known approach in the field concerned. The kit of this invention may contain the 1st antibody of the above, and the 2nd antibody of the above in one or the container beyond it. A kit may contain the explanation teaching materials in the sandwiches assay of SOBURINUSU which teach use of an antibody again. A kit may contain the suitable reagent for detecting the electropositive control for detection of an indicator, and electronegative control, a washing solution, the dilution buffer solution, etc.

[0045] As mentioned above, the 1st antibody is usually fixed by solid phase, and the indicator of the 2nd antibody is carried out. The 2nd antibody is made to react with SOBURINUSU by the liquid phase, and indicator-antibody-SOBURINUSU complex is made to form first in measurement of SOBURINUSU. And the reaction mixture containing this complex is made to react with the 1st antibody solidified as a mobile phase. Consequently, the 1st antibody and 2nd antibody are combined in the shape of sandwiches through SOBURINUSU. Therefore, only when SOBURINUSU exists, an indicator is fixed on solid phase through SOBURINUSU.

[0046] Trust number FERM The anti-SOBURINUSU monoclonal antibody produced by the BP-7202 No. cell strain has a high binding affinity in SOBURINUSU, and does not have a binding affinity to other SUTOREPUTOKKOKASU group bacteria. Trust number FERM Similarly, the anti-SOBURINUSU monoclonal antibody produced by the BP-7203 No. cell strain also has a high binding affinity in SOBURINUSU, and does not have a binding affinity as other SUTOREPUTOKKOKASU specieses.

[0047] The fragment of the functionality holding the joint property is also contained in the "anti-SOBURINUSU monoclonal antibody" by this invention. These fragments can compete about the specific binding to the intact antibody and intact SOBURINUSU in which they originate, and can be combined with the compatibility of at least 107, 108, 109M-1, or 1010M-1. The fragment of an antibody may contain the heavy chain of an immunoglobulin, a light chain, Fab, Fab', 2 (ab'), Fabc, and Fv. The fragment of an antibody may be produced according to separation of an intact immunoglobulin chemical [like an enzyme]. For example, F(ab')2 fragment is Harlow and Lane, and ANTIBODIES:A. LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, New It can obtain from an IgG molecule using a standard approach which was indicated by York (1988) by carrying out protein digestion by the

pepsin in pH 3.0-3.5. A Fab fragment can be obtained from all antibodies by restrictive reduction by the bottom papain digestion of existence of F(ab')2 fragment to a reducing agent (refer to the volume Paul and on W., 2nd edition RavanPress of FUNDAMENTAL IMMUNOLOGY, N.Y., 1989, and Chapter 7).

[Translation done.]

* NOTICES *

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3.In the drawings, any words are not translated.

EXAMPLE

[Example] Hereafter, it explains still more concretely about production of the monoclonal antibody production cell strain of this invention. This invention is not limited by the following examples.
[0049] In the example below <enzyme immunoassay (the ELISA method)>, evaluation of the obtained antiserum, a culture supernatant, and a monoclonal antibody was performed by the ELISA method. The operation information is indicated below.
[0050] (A) Coating SOBURINUSU of an antigen (SOBURINUSU) was prepared to 108 cells / concentration of mL. 100microl / well impregnation was carried out to the microplate (high [made from polystyrene] joint mold flat bottom # 2580, coaster company make), and the antigen solution was saved in saturated steam at the room temperature overnight at it. The aspirator removed the antigen solution just before the experiment.
[0051] (B) 200microl / well impregnation of 1 % of the weight BSA-PBS-Az (Az: aza-id sodium salt) of blocking were carried out, and it was left at the room temperature for 30 minutes. Then, the aspirator removed 1 % of the weight BSA-PBS-Az. When not conducting subsequent experiments on the same day, it is in this condition and saved at 4 degrees C in saturated steam.
[0052] (C) The antibody solutions (antiserum, a culture supernatant, purification antibody, etc.) diluted to concentration various by 1 % of the weight BSA-PBS-Az of reactions of an antibody were poured in by 50microl / well, and 1 % of the weight BSA-PBS-Az was poured in by 50microl / well. After half[1 hour and]-leaving it in ordinary temperature, PBS which washes 3 times by PBS and remains with an aspirator was removed.
[0053] (D) 50microl / well impregnation of the thing which dissolved the anti-mouse IgG antibody (product made from KPL) of the goat origin in which 0.2micro g/mL of reactions of the 2nd antibody carried out peroxidase labeling in the PBS solution of BSA 1% of the weight, or the thing which dissolved the anti-mouse IgM antibody (product made from KPL) of the goat origin in which 0.2microg/mL carried out peroxidase labeling in the PBS solution of BSA 1% of the weight were carried out, and it was left in ordinary temperature for 30 minutes. PBS which washes 3 times by PBS and remains with an aspirator further was removed.
[0054] (E) The reaction of a substrate and halt O-phenylenediamine (for biochemistry) 40mg were dissolved in the citric-acid-phosphoric-acid buffer (pH5) of 10mL(s), 100microl / well impregnation of the solution (substrate solution) which added 4micro of hydrogen peroxide solution L 30% of the weight just before use were carried out, and room temperature neglect was carried out. After about 3 minutes, 25microl / well impregnation of the 4-N sulfuric acid were carried out, and the reaction was suspended.
[0055] (F) The absorbance of 492nm was measured using the measurement microplate reader (Oriental soda company make).
[0056] In addition, although enzyme immunoassay was used as immunoassay in this example, the RIA method, a fluorescent antibody technique, etc. may be used for others.
[0057] In <example> this example, the BALB/C network mouse was used for immunity, taking that there is a track record at this invention persons' lab, and that a BALB/C network mouse is most often used in the antibody mass culture by the ascites after monoclonal antibody production cell strain establishment into consideration.
[0058] (Immunity) SOBURINUSU which is immunogen was prepared to 108 cells / mL using the physiology salt concentration phosphate buffer solution (PBS). The adjuvant emulsion containing immunogen was obtained by adding the adjuvant (a Homo sapiens tubercular killed bacteria content complete Freund's adjuvant, the Wako Pure Chem make, H37Rv) of this volume in the PBS solution of this SOBURINUSU, and fully emulsifying by engine-speed 1000rpm by the homogenizer in it.
[0059] 15 mice (BALB/C) of the female of about seven weeks of after the birth were injected with immunogen, and intraperitoneal or hypodermically were injected with the **** adjuvant emulsion every [100micro / l]. The

incomplete Freund's adjuvant of the SOBURINUSU solution and this which were prepared to 108 cells / mL using PBS, and this volume was emulsified by the homogenizer after two weeks, and the part same to a BALB/C mouse as last time was injected with this emulsion every [100micro / l].

[0060] Then, the mouse was injected with imperfect furoin tor undergarment TOEMARUJON which contains SOBURINUSU of the same presentation as the immunity of two weeks after, and concentration after four weeks, six weeks, and half a year from immunity initiation to the 100micro part as last time same [every / l]. It collected blood the one-week back of the 2nd injection, and after [of the 5th injection] one week, respectively, and the antibody production shown below was checked.

[0061] (Check of an antibody production) The blood serum was separated from the extracted blood and the antibody production was checked with enzyme immunoassay (the ELISA method) using the obtained blood serum. The 108 cells / mL, and SOBURINUSU prepared by PBS-Az as solid phase were poured distributively 100microl / well every, and the microplate which carried out the coat at the room temperature overnight was used. The peroxidase-labeling anti-mouse IgG antibody or the peroxidase-labeling anti-mouse IgM antibody was used as the second antibody. It is checked that the antibody combined with SOBURINUSU exists in an antibody sample by coloring in a well.

[0062] Consequently, production of an anti-SOBURINUSU antibody was accepted in the mice of all 15 animals. Furthermore, also in which mouse, it was checked that the antibody production has shifted from IgG to IgM after the 2nd injection, and after injection which is the 5th time, an IgG/IgM ratio is 300 or more and it checked that the class switching had happened enough.

[0063] (Cell fusion) The last immunity was performed in order to hypertrophy the spleen of three animals which was high as for especially the potency in the mouse which carried out immunity. SOBURINUSU of immunogen was prepared to 108 cells / concentration of mL six months after immunity initiation using PBS, and the mouse was injected every [100micro / l], without adding an adjuvant.

[0064] The spleen cell of one animal was extracted among the mice which passed three days after the last immunity. Using the polyethylene glycol of a mean molecular weight 1,500, with the conventional method, the spleen cell and the mouse myeloma origin cell strain (P3X63 Ag8.653) were united, and syncytium was obtained.

[0065] It wound around one 96 well plate, after making the hypoxanthine / aminopterin / thymidine (HAT) culture medium which prepared syncytium by the ISHIKOFU culture medium containing 15% of the weight of fetal calf serum (following, FCS) float (200microl / well). Under the present circumstances, the feeder cell (cell which supplies a growth factor at the time of culture initiation) used the spleen cell of the same mouse individual. Culture was started within the CO2 incubator (CO2 concentration: five volume %, temperature:37 degree C, humidity:95%). By the following culture, unless it was shown in others, it cultivated on the same conditions as this.

[0066] (Cell sorting and cloning) one -- a week -- after -- syncytium -- a culture supernatant -- 100 -- micro -- l -- extraction -- having carried out -- after -- syncytium -- containing -- the remainder -- culture medium -- four -- a sheet -- 24 -- a well -- a plate -- a passage -- carrying out -- each -- a well -- one -- ml -- 15 -- % of the weight -- FCS -- containing -- hypoxanthine -- /-- thymidine -- (-- HT --) -- a culture medium -- having added .

[0067] Four days after carrying out the passage of the syncytium to 24 well plate, cell culture supernatant liquid was extracted 150microl / well every. The binding affinity to SOBURINUSU was measured by the ELISA method shown below using this culture supernatant and the culture supernatant extracted after culture initiation at the 1st week.

[0068] SOBURINUSU prepared to the concentration of 5microg/mL by 0.1 mg/mL-BSA-PBS-Az as solid phase was used 100microl / well every. Cell culture supernatant liquid was used as antibody liquid. The peroxidase-labeling anti-mouse IgG antibody was used as the 2nd antibody.

[0069] The ELISA method result of the culture supernatant extracted twice was doubled, and 20 good wells of a vegetative state which have a high binding affinity to SOBURINUSU were checked. As the 1st-step selection, the passage of all of the cell of these wells was carried out to four 6 well plates, and the **** HT culture medium was added for 15% of the weight of 4ml FCS to each well.

[0070] The culture supernatant was extracted two days after cell sorting of the 1st step, and the binding affinity to SOBURINUSU was measured by the ELISA method shown below.

[0071] SOBURINUSU prepared to 108 cells / concentration of mL by PBS-Az as solid phase was used 100microl / well every. Cell culture supernatant liquid was used as antibody liquid. As the second antibody, the peroxidase-labeling anti-mouse IgG antibody was used.

[0072] Consequently, 10 well sorting of the well with a high binding affinity was carried out to SOBURINUSU. The passage of the cell of these wells was carried out to the inside flask (capacity of 50ml), respectively. The culture medium added 45ml of HT culture media containing 15% of the weight of FCS at a time.
[0073] The culture supernatant was extracted passage days [three days] after the cell which received the 2nd-step selection, and the binding affinity to SOBURINUSU and other SUTOREPUTOKKOKASU kinds (streptococcus mu factor wardrobe) was measured by the ELISA method shown below.
[0074] As solid phase, the mu factor wardrobe prepared to 108 cells / concentration of mL by PBS-Az was used 100microl / well every. Cell culture supernatant liquid was used as antibody liquid. As the second antibody, the peroxidase-labeling anti-mouse IgG antibody was used. If the antibody combined with a mu factor wardrobe exists in cell culture supernatant liquid, it will color on a well.
[0075] Only SOBURINUSU showed the binding affinity and 2 well sorting of the well which does not show a binding affinity to the mu factor wardrobe which are other SUTOREPUTOKKOKASU group bacteria was carried out.
[0076] About the cell of the two above-mentioned well, using HT culture medium containing 15% of the weight of FCS, it diluted to the concentration in which two cells per one well are contained (limiting dilution), and poured distributively each to two microplates of 96 wells. Initial growth was urged using the thymocyte of the mouse (BALB/C) of the female of four weeks of after the birth as a feeder. Culture was advanced raising the size of a plate and screening by the above-mentioned ELISA method was repeated about cell culture supernatant liquid timely. Finally the cell strain in which a high potency is shown and good growth is shown to SOBURINUSU was sorted out, and culture was advanced until it resulted in 5x105 cells / concentration of mL in the culture medium of 200mL. Finally, two shares of stocks which have a high binding affinity and do not start a cross reaction to other SUTOREPUTOKKOKASU group bacteria to SOBURINUSU were selected.
[0077] One share of the inside which showed the high binding affinity to SOBURINUSU was named cell strain name:SS-1, and domestic deposition was carried out in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on June 30, Heisei 12 (trust number FERM BP No. 7202).
[0078] One more share with a high binding affinity was similarly named cell strain name:SS-2 to SOBURINUSU, and domestic deposition was carried out in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on June 30, Heisei 12 (trust number FERM BP No. 7203).
[0079] The antibody (SS-1 share and SS-2 share) to produce is called SS1 antibody and SS2 antibody, respectively.
[0080] (Preservation of a cell) After it carried out centrifugal separation of the cell strain finally sorted out, and it removed supernatant liquid, made solution 1mL of FCS:dimethyl sulfoxide =9:1 (volume ratio) float by 1x107 cells / concentration of mL and carried out preliminary freezing at -80 degrees C, it was moved into liquid nitrogen and changed into the mothball condition.
[0081] (Purification of an antibody) Mass culture of the two selected shares was carried out by the ISHIKOFU culture medium which contains FCS 15% of the weight, respectively, and centrifugal separation of the supernatant liquid was carried out. Moreover, inject intraperitoneal [of a female BALB/C mouse] with two selected shares, respectively, it was made to increase, and ascites was stored up. The accumulated ascites was extracted. The culture supernatant or ascites of each stock was poured on the affinity chromatography using protein A joint gel (protein A sepharose 4FF, Pharmacia manufacture), and each monoclonal antibody (SS1 antibody and SS2 antibody) was refined on condition that the following.
[0082] The column filled up with protein A joint gel was equilibrated with the joint buffer solution (a 1.5M glycine and 3M NaCl, pH8.9). After diluting a culture supernatant or ascites with the joint buffer solution about 3 times, it was applied to the column which equilibrated. The joint buffer solution washed the column until the elution of an impurity was completed acting as the monitor of the eluate from a column by 280nm. The elution buffer solution (a 100mM citric acid, pH4) was applied to the column after washing (linear flow rate : about 20cm/(hour)), and IgG content eluates were collected. About the collected IgG content eluate, the absorbance of 280nm was measured with the absorptiometer and the concentration of an antibody was determined by converting the measured absorbance with an absorbancy index.
[0083] From the comparison with the standard protein by SDS polyacrylamide gel electrophoresis, each purification fractionation of these monoclonal antibodies (SS1 and SS2) checked that it was IgG which consists of molecular weight about 50,000 H chain, and about 25,000 L chain. In addition, mixing of an impurity was below limit of detection on electrophoresis.
[0084] (Evaluation of an antibody) About two kinds of monoclonal antibodies refined by the above-mentioned

affinity chromatography, antibody evaluation was performed using the dilution sequence of SOBURINUSU on the same conditions as the inhibition ELISA method in the 2nd-step [above-mentioned] selection.

[0085] Drawing 1 is a graph which shows the result of having measured the binding affinity [respectively as opposed to / antibody / SS1 / similarly / antibody / SS2 / (a) SOBURINUSU (white round head) and (b) mu factor wardrobe (black dot) in drawing 2].

[0086] As shown in drawing 1 , by SS1 antibody, to association to SOBURINUSU having been observed with x1 - x1/10 diluent, the mu factor wardrobe was received, and association was not observed about the diluent of a gap, either, but it was shown that SOBURINUSU can be specifically detected by SS1 antibody.

[0087] Similarly, as shown in drawing 2 , the mu factor wardrobe was received to association to SOBURINUSU having been observed with x1 - x1/100 diluent also in SS2 antibody, and association was not observed about the diluent of a gap, either, but it was shown that SOBURINUSU can be specifically detected by SS2 antibody.

[0088] (Sandwiches reaction) In the ELISA method, the coat of the SS1 antibody is carried out to a plate, SOBURINUSU was combined, after making SS2 antibody which carried out the enzyme label react, the excessive antibody was removed, and sufficient coloring was obtained, when the chromophoric substrate was added and having been incubated. That is, the combination of SS1 antibody, and an SS2 antibody or a polyclonal antibody has checked the useful thing to the inspection approach using sandwiches reactions, such as an immunity chromatography.

[0089] (Detection sensitivity in an immunity chromatography) SS2 antibody which fixed and carried out the gold colloid indicator of the SS1 antibody on the filter paper according to the conventional method was put on the mobile phase, and immunity chromatography equipment was produced. When the sample which contains SOBURINUSU by various concentration was applied, the sensibility of this immunity chromatography equipment was about 106 cells / ml.

[0090] Generally, it is known that the SOBURINUSURE bells in healthy people's inside of the oral cavity are about 104 to 107 cell / ml. Therefore, the immunity chromatography equipment produced using the antibody of this invention can detect SOBURINUSU in the inside of the oral cavity.

[Translation done.]

* NOTICES *

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the graph using SS1 antibody which shows the result of the ELISA method. The data of a graph show the binding affinity to SOBURINUSU and the mu factor wardrobe of an anti-SOBURINUSU monoclonal antibody (SS1 antibody) of this invention.
[Drawing 2] It is the graph using SS2 antibody which shows the result of the ELISA method. The data of a graph show the binding affinity to SOBURINUSU and the mu factor wardrobe of an anti-SOBURINUSU monoclonal antibody (SS2 antibody) of this invention.

[Translation done.]

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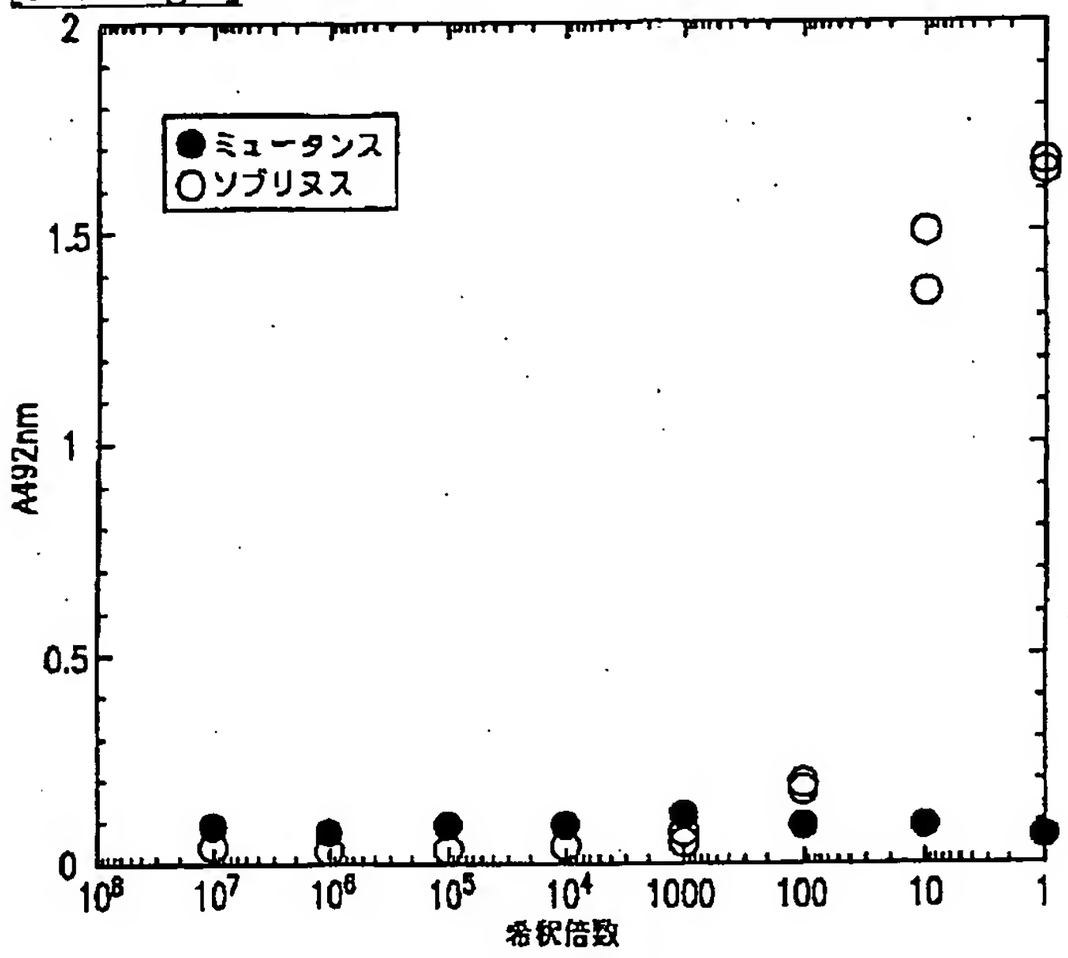
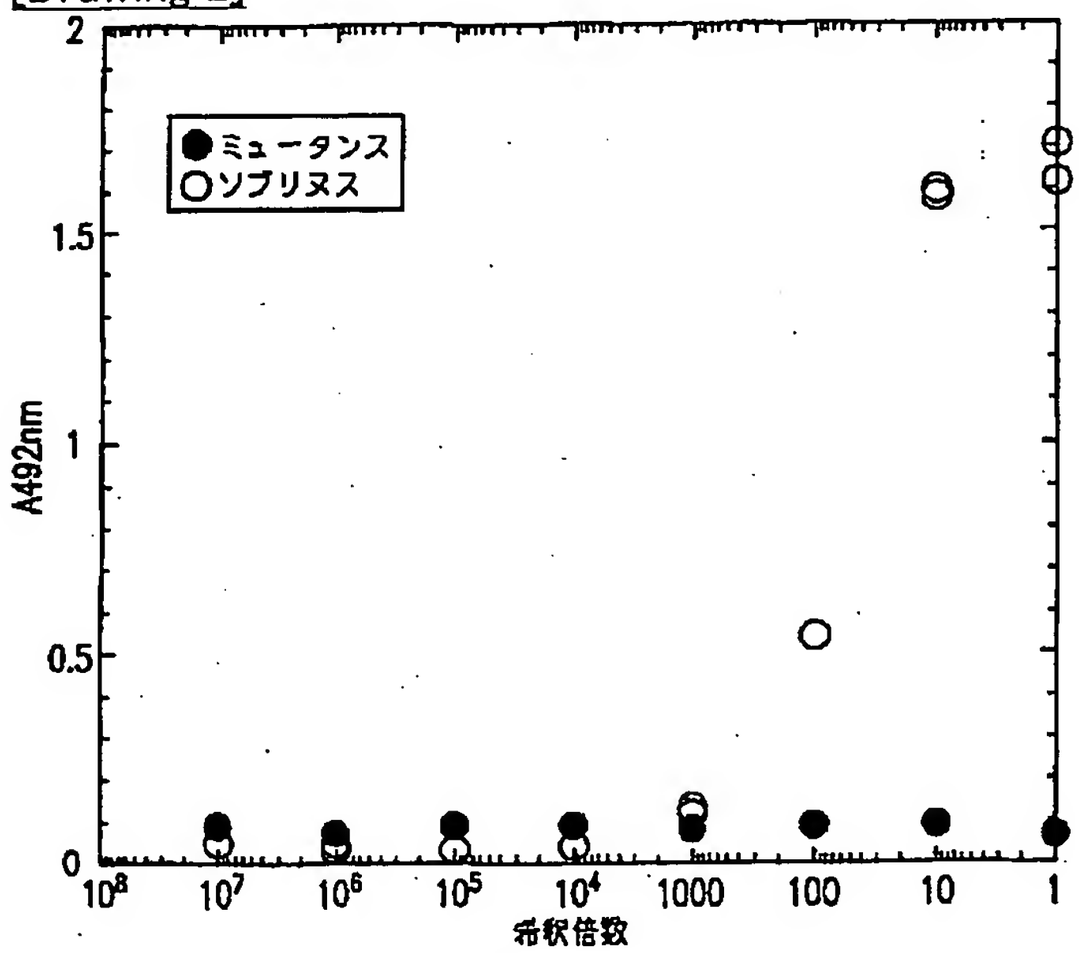
DRAWINGS

[Drawing 1]



[Drawing 2]



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CA25 CA46
4H045 AA11 CA40 DA76 EA50 FA72

(54)【発明の名称】　抗ソブリヌスモノクローナル抗体、およびそれを産生する細胞株またはそれを含むソブリヌス検出用キット

(57)【要約】
【課題】　ソブリヌスの定量を正確かつ簡便に行う手段を提供する。
【解決手段】　ソブリヌスに特異的なモノクローナル抗体を産生する細胞株であって、ソブリヌスで免疫した哺乳動物の脾臓細胞と、哺乳動物の骨髄腫由来の細胞とを細胞融合して、融合細胞を得る工程；および、ソブリヌスに対して特異的なモノクローナル抗体を産生する細胞株を該融合細胞の中からクローニングする工程であって、該融合細胞から産生される抗体を、ソブリヌスおよびその他のストレプトッコカス属細菌に対する結合能について免疫測定法により検定する工程を包含する方法により得られる。

【特許請求の範囲】
【請求項１】　ソブリヌスに特異的なモノクローナル抗体を産生する細胞株であって、
ソブリヌスで免疫した哺乳動物の脾臓細胞と、哺乳動物の骨髄腫由来の細胞とを細胞融合して、融合細胞を得る工程；および、
ソブリヌスに対して特異的なモノクローナル抗体を産生する細胞株を該融合細胞の中からクローニングする工程であって、該融合細胞から産生される抗体を、ソブリヌスおよびその他のストレプトッコカス属細菌に対する結合能について免疫測定法により検定する工程を包含する方法により得られる、細胞株。
【請求項２】　工業技術院生命工学工業技術研究所受託番号ＦＥＲＭ　ＢＰ－７２０２号、またはＦＥＲＭ　ＢＰ－７２０３号である、請求項１に記載のモノクローナル抗体産生細胞株。
【請求項３】　前記哺乳動物の骨髄腫由来の細胞が、マウス骨髄腫由来Ｐ３Ｘ６３　Ａｇ８．６５３株の細胞である、請求項１に記載の細胞株。
【請求項４】　前記免疫測定法が、酵素免疫測定法（ＥＬＩＳＡ法）である、請求項１に記載の細胞株。
【請求項５】　前記免疫した哺乳動物が、マウスまたはラットである、請求項１に記載の細胞株。
【請求項６】　前記免疫した哺乳動物が、ＢＡＬＢ／Ｃ系統マウスである、請求項１に記載の細胞株。
【請求項７】　請求項1に記載の細胞株により産生される抗ソブリヌスモノクローナル抗体であって、ソブリヌスに高い結合能を有し、その他のストレプトッコカス属細菌に結合能を有しない、モノクローナル抗体。
【請求項８】　ソブリヌスを検出するためのキットであって、固相に結合された第１の抗体、および第２の抗体を含み、該第１の抗体が請求項７に記載のモノクローナル抗体であり、そして該第２の抗体がソブリヌスに特異的に結合する標識抗体である、キット。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明は、ソブリヌス菌（Ｓｔｒｅｐｔｏｃｏｃｃｕｓ　ｓｏｂｒｉｎｕｓ、以下単にソブリヌスと記す）に特異的なモノクローナル抗体、およびこのモノクローナル抗体を産生する細胞株、およびこのモノクローナル抗体を含むキットに関する。
【０００２】
【従来の技術】ソブリヌスは、ミュータンス菌（Ｓｔｒｅｐｔｏｃｏｃｃｕｓ　ｍｕｔａｎｓ、以下単にミュータンスと記す）とともに、動物の口腔内に生息し虫歯の原因となる細菌である。そのため、ソブリヌスの検査は、虫歯予防の観点から非常に有用である。例えば、ソブリヌスに対して特異的な抗体が得られれば、虫歯予防検査、ソブリヌス量の定量による病態スクリーニング、治療効果の確認、および予後の判定等を行なうことが出

来る。しかし、従来、ソブリヌスに対して特異的な抗体は得られておらず、そしてソブリヌスを特異的に検出する方法はなく、一般的には、培養法を利用して、他のミュータンスグループの細菌（Ｓ．ｍｕｔａｎｓ、Ｓ．ｒｉｃｅｔｕｓ、Ｓ．ｒａｔｔｕｓなど）とともに生育させ、生育したミュータンスグループの細菌集団の中に存在するソブリヌスを同定および検出するという検査方法が実施されていた。
【０００３】しかし、培養法を用いたソブリヌスの検出のためには、２４時間以上の時間を必要とし、操作が煩雑で、またソブリヌスの定量も容易ではない。そのため、ソブリヌスの定量をより正確かつ簡便に行うことが望まれてきた。
【０００４】
【発明が解決しようとする課題】本発明は、ソブリヌスの定量を正確かつ簡便に行う手段を提供することを目的とする。本発明は、ソブリヌス菌に特異的なモノクローナル抗体を提供し、これを用いたサンドイッチ方式の免疫クロマトグラフィーによる簡便なソブリヌスの定量法を提供する。
【０００５】ソブリヌスは、細菌であり、ソブリヌスを介してサンドイッチ状に結合できる抗体のペアを得ることは比較的容易である。しかし、モノクローナル抗体を用いる場合、抗体が均一であるため、このモノクローナル抗体の交叉反応性が非常に重要である。ソブリヌスに対するモノクローナル抗体の中には、ソブリヌスだけでなく他のストレプトッコカス属細菌に対しても高い結合能を有する抗体が存在し得る。このような抗体をサンドイッチ法に用いると、ソブリヌスの正確な定量に支障をきたす恐れがある。従って、他のストレプトッコカス属細菌に対する交叉反応性が低い抗ソブリヌスモノクローナル抗体を用いる必要がある。
【０００６】本発明は、上記問題点の解決を意図するものであり、ソブリヌスに対して高い結合能を有するが、他のストレプトッコカス属細菌に対する交叉反応性が低い抗体、およびこれを産生する細胞株、ならびにこの抗体を含むソブリヌス検出用キットを提供することを目的とする。
【０００７】
【課題を解決するための手段】本発明は、ソブリヌスに特異的なモノクローナル抗体を産生する細胞株に関し、この細胞株は、ソブリヌスで免疫した哺乳動物の脾臓細胞と、哺乳動物の骨髄腫由来の細胞とを細胞融合して、融合細胞を得る工程；およびソブリヌスに対して特異的なモノクローナル抗体を産生する細胞株を該融合細胞の中からクローニングする工程であって、上記融合細胞から産生される抗体を、ソブリヌスおよびその他のストレプトッコカス属細菌に対する結合能について免疫測定法により検定する工程を包含する方法により得られる。
【０００８】好ましくは、モノクローナル抗体産生細胞

株は、工業技術院生命工学工業技術研究所受託番号ＦＥＲＭ　ＢＰ－７２０２号、またはＦＥＲＭ　ＢＰ－７２０３号である。

【０００９】好ましくは、上記哺乳動物の骨髄腫由来の細胞は、マウス骨髄腫由来Ｐ３Ｘ６３　Ａｇ８．６５３株の細胞である。好ましくは、上記免疫測定法は、酵素免疫測定法（ＥＬＩＳＡ法）である。

【００１０】好ましくは、上記免疫した哺乳動物は、マウスまたはラットである。

【００１１】好ましくは，上記免疫した哺乳動物は、ＢＡＬＢ／Ｃ系統マウスである。

【００１２】本発明は、１つの局面で、上記細胞株により産生される抗ソブリヌスモノクローナル抗体に関し、このモノクローナル抗体は、ソブリヌスに高い結合能を有し、その他のストレプトッコカス属細菌に結合能を有しない。

【００１３】本発明は、１つの局面で、ソブリヌスを検出するためのキットに関し、このキットは、固相に結合された第１の抗体、および第２の抗体を含み、この第１の抗体は、上記モノクローナル抗体であり、そして上記第２の抗体はソブリヌスに特異的に結合する標識された抗体である。

【００１４】上記第２の抗体は、上記細胞株により産生される抗ソブリヌスモノクローナル抗体であり得るか、または、ソブリヌスに対して高い結合能を有する他の抗体であり得る。

【００１５】

【発明の実施の形態】以下、本発明について、より具体的に説明する。

【００１６】本発明においては、特に指示のない限り、当該分野で公知である、タンパク質の分離および分析法、ならびに免疫学的手法が採用され得る。これらの手法は、市販の酵素、キット、抗体、標識物質などを使用して行い得る。

【００１７】本発明の抗ソブリヌスモノクローナル抗体産生細胞株の作製を、以下に作製手順に沿って説明する。

【００１８】（免疫）まず、哺乳動物をソブリヌスで免疫することによって、動物体内で抗体産生細胞を調製する。

【００１９】「哺乳動物」の例として、マウス、ラット、ウシ、ウサギ、ヤギ、ヒツジ、モルモットが挙げられる。哺乳動物は、好ましくはマウスおよびラットであり、より好ましくはマウスである。マウスの例として、Ａ／Ｊ系統、ＢＡＬＢ／Ｃ系統、ＤＢＡ／２系統、Ｃ５７ＢＬ／６系統、Ｃ３Ｈ／Ｈｅ系統、ＳＪＬ系統、ＮＺＢ系統、ＣＢＡ／ＪＮＣｒｊ系統のマウスが挙げられる。ＢＡＬＢ／Ｃ系統のマウスは、免疫後に血清中に高い抗体力価を示すので、ソブリヌスとの親和性が極めて高いモノクローナル抗体を得ることが可能である。血中

抗体力価が、特異的なハイブリドーマの出来易さと関係していることは公知である。また、細胞株の確立後の腹水による抗体大量作製においては、ＢＡＬＢ／Ｃ系統マウスが一般によく使用される。以上により、ＢＡＬＢ／Ｃ系統のマウスは、ソブリヌスの免疫に好ましい例である。実験動物の齢は、特に限定されないが、代表的には約４週齢～約１２週齢であり、好ましくは約６～約１０週齢、より好ましくは約７週齢のマウスまたはラットである。

【００２０】免疫に用いられるソブリヌスは、人の口腔内から単離してもよいし、市販（例えば、アメリカンタイプカルチャーコレクション、アメリカ合衆国　メリーランド　２０８５２、ロックビル、パークローン　ドライブ　１２３０１から入手し得る）のものを用いてもよい。人の口腔内からは、定法に従い、ＭＳ寒天培地上でムコイド型のコロニーとして単離され得る。

【００２１】免疫の前に、ソブリヌスは、免疫応答を増強させるためにアジュバントと混合され得る。アジュバントの例としては、油中水型乳剤（例えば、不完全フロイントアジュバント）、水中油中水型乳剤、水中油型乳剤、リポソーム、水酸化アルミニウムゲル、シリカアジュバント、粉末ベントナイト、およびタピオカアジュバントの他に、ＢＣＧ、Ｐｒｏｐｉｏｎｉｂａｃｔｅｒｉｕｍ　ａｃｎｅｓなどの菌体、細胞壁およびトレハロースダイコレート（ＴＤＭ）などの菌体成分；グラム陰性菌の内毒素であるリポ多糖体（ＬＰＳ）およびリピドＡ画分；β　グルカン（多糖体）；ムラミルジペプチド（ＭＤＰ）；ベスタチン；レバミゾールなどの合成化合物；胸腺ホルモン、胸腺ホルモン液性因子およびタフトシンなどの生体成分由来のタンパク質またはペプチド性物質；ならびにそれらの混合物（例えば、完全フロイントアジュバント）などが挙げられる。

【００２２】これらのアジュバントは、投与経路、投与量、投与時期などに依存して免疫応答の増強または抑制に効果を示す。さらにアジュバントの種類によって、抗原に対する血中抗体産生、細胞性免疫の誘導、免疫グロブリンのクラスなどに差が認められる。それゆえ、目的とする免疫応答に応じて、アジュバントを適切に選択することが好ましい。選択されたアジュバントの取扱い、例えばソブリヌスとの混合方法などは当該分野で公知である。

【００２３】哺乳動物の免疫は、当該分野で公知の方法に従って行われる。例えば、抗原であるソブリヌスは、哺乳動物の皮下、皮内、静脈、または腹腔内に注射され得る。免疫応答は、免疫される哺乳動物の種類および系統によって異なるので、免疫スケジュールは、使用される動物に合わせて適切に変更され得る。抗原投与は、最初の免疫の後に、何回か繰り返される。追加免疫は、例えば、最初の免疫から４週間後、６週間後、および半年後に行われ得る。

【００２４】（抗体産生の確認）免疫後、哺乳動物から採血し、得られた血液をソブリヌス結合活性の存在についてアッセイすることにより、哺乳動物の体内でソブリヌスに対する抗体が産生されていること、および免疫末期にはＩｇＭからＩｇＧへのクラススイッチが起こっていることを確認する（例えば、ＨａｒｌｏｗおよびＬａｎｅ、ＡＮＴＩＢＯＤＩＥＳ：　Ａ　ＬＡＢＯＲＡＴＯＲＹ　ＭＡＮＵＡＬ、ＣＯＬＤ　ＳＰＲＩＮＧ　ＨＡＲＢＯＲ　ＬＡＢＯＲＡＴＯＲＹ、Ｎｅｗ　Ｙｏｒｋ（１９８８）を参照のこと）。適切なアッセイ方法の例として、酵素免疫測定法（ＥＬＩＳＡ法）、放射免疫アッセイ法（ＲＩＡ）、蛍光抗体法が挙げられる。本発明では、ソブリヌスに対して高親和性を有する抗ソブリヌスモノクローナル抗体を得ることが望ましい。高親和性のモノクローナル抗体産生細胞を得るためには、抗血清の時点で高い抗体価を示している必要がある。

【００２５】（ブースト）ソブリヌス結合性抗体の産生を確認した後、脾臓を肥大させるために、ブースト（免疫原の追加注射）を行い得る。ブーストで投与されるソブリヌスの量は、最初に免疫されるソブリヌスの量の約４～５倍の量が望ましいがこれに限定されない。

【００２６】ブーストは、代表的には、ソブリヌスと不完全フロイントアジュバントとのエマルジョンを用いて行われる。ただし、最終免疫（細胞融合数日前の免疫原の追加注射）で投与されるソブリヌスとしては、アジュバントを加えず純粋品を用いることが好ましい。投与経路は、皮下、皮内、静脈、または腹腔内それぞれの投与によって、ソブリヌスの異なった部位を認識する抗体が得られる可能性があることを考慮して、適宜決定される。

【００２７】（細胞融合）最終免疫後、免疫した哺乳動物から脾臓細胞を摘出し、骨髄腫由来の細胞株の細胞と細胞融合する。

【００２８】融合細胞の増殖能力は、細胞融合時に用いられる骨髄腫由来の細胞株の種類に依存するので、細胞融合には、増殖能力の優れた細胞株を用いることが好ましい。また、骨髄腫由来の細胞株は、融合する脾臓細胞の由来する哺乳動物と適合性があることが好ましい。骨髄腫由来の細胞株は、新たに調製してもよいし、市販のものを使用してもよい。マウスの骨髄腫由来の細胞株としては、Ｐ３Ｘ６３Ａｇ８．６５３、Ｓｐ２／０　Ａｇ１４、ＦＯ・１、Ｓ１９４／５．ＸＸ０　ＢＵ．１、Ｐ３／ＮＳ１／１　Ａｇ４　１などが挙げられる。抗体の断片を産生せず、かつ融合細胞の増殖能力が優れたものとなるため、Ｐ３Ｘ６３　Ａｇ８．６５３の使用が好ましい。ラット骨髄腫由来の細胞株としては、２１０、ＲＣＹ３．Ａｇ．１．２．３、ＹＢ２／０などが挙げられる。

【００２９】細胞融合は、当該分野で公知の方法に従って行われる（ＫｏｅｈｌｅｒおよびＭｉｌｓｔｅｉｎ、

Ｎａｔｕｒｅ　２５６：４９５［１９７５］、Ｋｏｓｂｏｒら、１９８３、Ｉｍｍｕｎｏｌ．　Ｔｏｄａｙ　４：７２、Ｃｏｔｅら、１９８３、Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ、８０：２０２６、Ｃｏｌｅら、ＭＯＮＯＣＬＯＮＡＬ　ＡＮＴＩＢＯＤＩＥＳ　ＡＮＤ　ＣＡＮＣＥＲＴＨＥＲＡＰＹ、Ａｌａｎ　Ｒ　Ｌｉｓｓ　Ｉｎｃ．、Ｎｅｗ　Ｙｏｒｋ、ＮＹ、７７－９６頁［１９８５］などを参照のこと）。細胞融合法の例として、例えば、ポリエチレングリコール法、センダイウイルスを用いた方法、電流を利用する方法などが挙げられる。細胞毒性も比較的少なく、融合操作も容易で再現性が高いため、ポリエチレングリコール法が好ましい。

【００３０】得られた融合細胞は、当該分野で公知の条件に従って増殖させ得る。産生される抗体の結合能に基づいて、所望の融合細胞を選択し得る。

【００３１】（細胞選別およびクローニング）融合細胞から産生される抗体の結合能は、当該分野で公知の方法に基づいてアッセイされ得る。本発明においては、ソブリヌスに高い結合能を有し、他のストレプトッコカス属細菌に対して結合能を有さないか、もしくは低い結合能を有する抗体を産生する融合細胞を得るために、他のストレプトッコカス属細菌に対する結合能に基づく選別を利用して、目的の細胞株をクローニングする。従って、本明細書中で用語「ソブリヌスに対して特異的なモノクローナル抗体」とは、ソブリヌスに高い結合能を有し、他のストレプトッコカス属細菌に対して結合能を有さないか、もしくは低い結合能を有する抗体を意味する。ソブリヌスに高い結合能を有し、他のストレプトッコカス属細菌に対して「結合能を有さない」抗体は、交叉反応性がない。

【００３２】用語「高い結合能を有する」とは、下記の実施例に記載されたインヒビションＥＬＩＳＡ法と実質的に同一の条件での測定においてインヒビションがかかり、インヒビションの半値が約10^{-6}Ｍ以下であることをいう。用語「低い結合能を有する」とは、同じ測定においてインヒビションがかかるが、インヒビションの半値が約10^{-5}Ｍ以上（例えば、10^{-4}Ｍ、10^{-3}Ｍなど）であることをいう。用語「結合能を有さない」とは、同じ測定においてインヒビションがかからないことをいう。用語「インヒビションがかかる」とは、固相に固定されたソブリヌスに結合する抗体の量が、競合物質（インヒビター）の存在下で、インヒビターの不存在下と比較して減少することをいう。用語「インヒビションがかからない」とは、固相に固定されたソブリヌスに結合する抗体の量が、インヒビターの存在下および不存在下で実質的に同等であることをいう。「インヒビションの半値」とは、インヒビターの不存在下での吸光度（抗体結合量を反映する）の半分の吸光度が測定されるインヒビターの濃度をいう。

【００３３】抗体の結合能は、抗体産生の確認に関して上述したのと同様に、ＥＬＩＳＡ法、ＲＩＡ法、蛍光抗体法などの方法を用いてアッセイされる。簡便に感度よく抗体を検出し得ることから、ＥＬＩＳＡ法が好ましい。

【００３４】融合細胞のクローニングには、当該分野で公知の方法が用いられ得る。クローニングの方法の例としては、限界希釈法、軟寒天法などが挙げられる。操作も容易で数多くの実績があり、再現性が高いため、限界希釈法が好ましい。

【００３５】細胞融合により得られた多くの融合細胞の中から、効率よく有用な細胞を選択するために、細胞選別は、クローニングの初期の段階から行うことが好ましい。

【００３６】このようにして、望ましい結合能を有する抗体を産生する融合細胞株が最終的に選別される。選別された細胞株は、液体窒素中で半永久的に保存され得る。

【００３７】（抗体の精製）上記のようにして選別されたモノクローナル抗体産生細胞株を大量培養することにより、ソブリヌスに対して特異的なモノクローナル抗体を大量に産生し得る。モノクローナル抗体産生細胞株の大量培養方法として、インビボおよびインビトロでの培養が挙げられる。インビボでの大量培養の例としては、哺乳動物の腹腔内に融合細胞を注射して増殖させ、腹水中に抗体を産生させる方法が挙げられる。インビトロでの培養では、融合細胞が培地中で培養され、抗体が培地中に産生される。

【００３８】大量培養により得られた腹水または培養上清から、当該分野で公知の方法を用いて、本発明のモノクローナル抗体を精製し得る。精製のためには、例えば、ＤＥＡＥ陰イオン交換クロマトグラフィー、アフィニティークロマトグラフィー、硫安分画法、ＰＥＧ分画法、エタノール分画法などが適宜組み合わせて用いられる。本発明の抗体は、通常、約９０％の純度、好ましくは約９５％の純度、より好ましくは約９８％の純度となるように精製される。

【００３９】（抗体の評価）精製されたモノクローナル抗体の結合能を評価することにより、得られたいくつかの抗体の中から、ソブリヌス細胞上の異なるエピトープを認識する抗体の組合せを選択し得る。異なるエピトープを認識する、任意の２種の抗体の組合せは、サンドイッチ法のために有用である。一方、細菌表面上には、同一のタンパク質が多く存在するため、一種類のモノクローナル抗体を使用してサンドイッチ法をすることも可能である。

【００４０】本発明においては、ソブリヌスを検出するためのキットが提供される。本発明のキットは、例えば、抗原抗体結合反応に基づいて水性試料中の抗原を検出する免疫クロマトグラフィーを実施するために提供され得る。本発明のキットは、固相に結合された第１の抗体および移動相に含められて用いられる第２の抗体を含む。

【００４１】固相に結合される第１の抗体としては、受託番号ＦＥＲＭ　ＢＰ－７２０２号または受託番号ＦＥＲＭ　ＢＰ－７２０３号の細胞株により産生されるモノクローナル抗体が使用され得る。好適には、受託番号ＦＥＲＭ　ＢＰ－７２０２号の細胞株により産生されるモノクローナル抗体が第１の抗体として使用され得る。

【００４２】第２の抗体としては、ソブリヌスに高い結合能を有する限り任意の抗体を使用し得、これは、ポリクローナル抗体であってもよいし、モノクローナル抗体であってもよい。好ましくはモノクローナル抗体が用いられる。第２の抗体として受託番号ＦＥＲＭ　ＢＰ－７２０２号または受託番号ＦＥＲＭ　ＢＰ－７２０３号の細胞株により産生されるモノクローナル抗体が好適に用いられる。この場合、受託番号ＦＥＲＭ　ＢＰ－７２０２号および受託番号ＦＥＲＭ　ＢＰ－７２０３号の細胞株により産生されるモノクローナル抗体を第１の抗体および第２の抗体の一組のペアとして用い、それによってサンドイッチアッセイを実施し得る。

【００４３】第２の抗体は、当該分野で公知の方法により任意の標識により標識され得る。標識の例としては、酵素標識、色素標識、磁性標識、放射性標識、色の付いた粒子（金コロイド、ラテックスなど）による標識などが挙げられる。

【００４４】本発明のキットは、当該分野で公知の方法により適切に作製され得る。本発明のキットは、１つまたはそれ以上の容器中に上記第１の抗体および上記第２の抗体を含み得る。キットはまた、ソブリヌスのサンドイッチアッセイにおける、抗体の使用を教示する説明教材を含み得る。キットは、標識の検出のため、または陽性コントロールおよび陰性コントロールを検出するための適切な試薬、洗浄溶液、希釈緩衝液などを含み得る。

【００４５】上記のように、第１の抗体は、通常、固相に固定化されており、そして第２の抗体は標識されている。ソブリヌスの測定にあたっては、まず、第２の抗体を液相でソブリヌスと反応させ、標識－抗体－ソブリヌス複合体を形成させる。そして、この複合体を含む反応液を移動相として固体化された第１の抗体と反応させる。その結果、第１の抗体および第２の抗体は、ソブリヌスを介してサンドイッチ状に結合する。従って、ソブリヌスが存在する場合にのみ、ソブリヌスを介して固相上に標識が固定化される。

【００４６】受託番号ＦＥＲＭ　ＢＰ－７２０２号の細胞株により産生される抗ソブリヌスモノクローナル抗体は、ソブリヌスに高い結合能を有し、他のストレプトッコカス属細菌には結合能を有しない。受託番号ＦＥＲＭ　ＢＰ－７２０３号の細胞株により産生される抗ソブリヌスモノクローナル抗体も同様に、ソブリヌスに高い結

合能を有し、他のストレプトッコカス種には結合能を有しない。

【００４７】本発明による「抗ソブリヌスモノクローナル抗体」には、その結合特性を保持した機能性の断片もまた含まれる。これらの断片は、それらが由来するインタクトな抗体とソブリヌスへの特異的結合について競合し得、少なくとも10^7、10^8、$10^9 M^{-1}$、または$10^{10} M^{-1}$の親和性で結合し得る。抗体の断片は、免疫グロブリンの重鎖、軽鎖、Ｆａｂ、Ｆａｂ’、（ａｂ’）$_2$、ＦａｂｃおよびＦｖを含み得る。抗体の断片は、インタクトな免疫グロブリンの酵素的または化学的分離によって生じ得る。例えば、Ｆ（ａｂ’）$_2$断片は、ＨａｒｌｏｗおよびＬａｎｅ、ＡＮＴＩＢＯＤＩＥＳ：ＡＬＡＢＯＲＡＴＯＲＹ　ＭＡＮＵＡＬ、ＣＯＬＤ　ＳＰＲＩＮＧ　ＨＡＲＢＯＲ　ＬＡＢＯＲＡＴＯＲＹ、Ｎｅｗ　Ｙｏｒｋ（１９８８）に記載されたような標準的な方法を用い、ｐＨ３．０～３．５においてペプシンでタンパク質消化することによってＩｇＧ分子から得ることができる。Ｆａｂ断片は、限定的還元によってＦ（ａｂ’）$_2$断片から、あるいは還元剤の存在下パパイン消化によって全抗体から得ることができる（Ｐａｕｌ、Ｗ．編、ＦＵＮＤＡＭＥＮＴＡＬ　ＩＭＭＵＮＯＬＯＧＹ第２版　ＲａｖａｎＰｒｅｓｓ、Ｎ．Ｙ．、１９８９、第７章を参照のこと）。

【００４８】

【実施例】以下、本発明のモノクローナル抗体産生細胞株の作製についてさらに具体的に説明する。本発明は以下の実施例によって限定されるものではない。

【００４９】＜酵素免疫測定法（ＥＬＩＳＡ法）＞以下の実施例においては、得られた抗血清、培養上清およびモノクローナル抗体の評価は、ＥＬＩＳＡ法により行なった。その操作法を以下に記載する。

【００５０】（Ａ）抗原（ソブリヌス）のコーティング

ソブリヌスを、10^8細胞／ｍＬの濃度に調製した。マイクロプレート（ポリスチレン製高結合型平底＃２５８０、コスター社製）に抗原溶液を１００μｌ／ウェル注入し、室温で飽和水蒸気中に一晩保存した。実験直前に、アスピレータで抗原溶液を除去した。

【００５１】（Ｂ）ブロッキング

１重量％ＢＳＡ－ＰＢＳ－Ａｚ（Ａｚ：アザイドナトリウム塩）を２００μｌ／ウェル注入し、３０分間室温で放置した。その後、アスピレータで１重量％ＢＳＡ－ＰＢＳ－Ａｚを除去した。以降の実験を即日に行わないときは、この状態で、飽和水蒸気中に４℃で保存した。

【００５２】（Ｃ）抗体の反応

１重量％ＢＳＡ－ＰＢＳ－Ａｚで種々の濃度に希釈した抗体溶液（抗血清、培養上清、精製抗体等）を５０μｌ／ウェル、および１重量％ＢＳＡ－ＰＢＳ－Ａｚを５０μｌ／ウェルで注入した。常温で１時間半放置した後、ＰＢＳで３回洗浄し、アスピレータで残存するＰＢＳを除去した。

【００５３】（Ｄ）第２抗体の反応

０．２μｇ／ｍＬのペルオキシダーゼ標識したヤギ由来の抗マウスＩｇＧ抗体（ＫＰＬ社製）を１重量％ＢＳＡのＰＢＳ溶液に溶解したもの、または０．２μｇ／ｍＬのペルオキシダーゼ標識したヤギ由来の抗マウスＩｇＭ抗体（ＫＰＬ社製）を１重量％ＢＳＡのＰＢＳ溶液に溶解したものを５０μｌ／ウェル注入し、常温で３０分放置した。ＰＢＳで３回洗浄し、さらにアスピレータで残存するＰＢＳを除去した。

【００５４】（Ｅ）基質の反応と停止

Ｏ－フェニレンジアミン（生化学用）４０ｍｇを１０ｍＬのクエン酸－リン酸バッファー（ｐＨ５）に溶解し、使用直前に３０重量％過酸化水素水４μＬを加えた溶液（基質溶液）を１００μｌ／ウェル注入し、室温放置した。約３分後、４Ｎ硫酸を２５μｌ／ウェル注入して反応を停止した。

【００５５】（Ｆ）測定

マイクロプレートリーダ（東洋ソーダ社製）を用いて４９２ｎｍの吸光度を測定した。

【００５６】なお、本実施例では免疫測定法として酵素免疫測定法を用いたが、他にＲＩＡ法、蛍光抗体法等を用いてもよい。

【００５７】＜実施例＞本実施例においては、本発明者らの研究所で実績があること、およびモノクローナル抗体産生細胞株確立後の腹水による抗体大量培養においてはＢＡＬＢ／Ｃ系統マウスが最もよく使用されることを考慮に入れ、ＢＡＬＢ／Ｃ系統マウスを免疫に使用した。

【００５８】（免疫）免疫原であるソブリヌスを、生理食塩濃度リン酸緩衝液（ＰＢＳ）を用いて10^8細胞／ｍＬに調製した。このソブリヌスのP.ＢＳ溶液に、同体積のアジュバント（ヒト結核死菌含有完全フロイントアジュバント、和光純薬製、Ｈ３７Ｒｖ）を添加し、ホモジナイザで回転数１０００ｒｐｍで充分に乳化することにより、免疫原を含むアジュバントエマルジョンを得た。

【００５９】生後約７週間の雌のマウス（ＢＡＬＢ／Ｃ）１５匹に、免疫原を含むアジュバントエマルジョンを１００μｌずつ腹腔内、あるいは皮下に注射した。２週間後、ＰＢＳを用いて10^8細胞／ｍＬに調製したソブリヌス溶液およびこれと同体積の不完全フロイントアジュバントをホモジナイザで乳化し、このエマルジョンをＢＡＬＢ／Ｃマウスに前回と同じ部位に１００μｌずつ注射した。

【００６０】その後、免疫開始より４週間後、６週間後、および半年後に、２週間後の免疫と同じ組成、濃度のソブリヌスを含む不完全フロイントアジュバントエマルジョンを、マウスに１００μｌずつ前回と同じ部位に注射した。２回目の注射の１週間後と5回目の注射の１

週間後にそれぞれ採血し、以下に示す抗体産生を確認した。

【００６１】（抗体産生の確認）採取した血液から血清を分離し、得られた血清を用いて、酵素免疫測定法（ＥＬＩＳＡ法）により抗体産生の確認をした。固相としてＰＢＳ－Ａｚで調製した10^8細胞／ｍＬ・ソブリヌスを、１００μｌ／ウェルずつ分注し、室温で一晩コートしたマイクロプレートを使用した。第二抗体としてペルオキシダーゼ標識抗マウスＩｇＧ抗体、またはペルオキシダーゼ標識抗マウスＩｇＭ抗体を使用した。ウェル中での発色により、抗体サンプル中にソブリヌスに結合する抗体が存在することが確認される。

【００６２】その結果、１５匹すべてのマウスにおいて抗ソブリヌス抗体の産生が認められた。さらに、いずれのマウスにおいても、２回目の注射後に抗体産生がＩｇＧからＩｇＭへシフトしていることが確認され、５回目の注射後にはＩｇＧ／ＩｇＭ比が３００以上でありクラススイッチが充分起こっていることを確認した。

【００６３】（細胞融合）免疫したマウスの中で特に力価の高かった３匹の脾臓を肥大させるために、最終免疫を行なった。免疫開始から６ヶ月後、免疫原のソブリヌスを、ＰＢＳを用いて10^8細胞／ｍＬの濃度に調製し、アジュバントを加えずにマウスに１００μｌずつ注射した。

【００６４】最終免疫後３日を経過したマウスのうち１匹の脾臓細胞を摘出した。平均分子量１，５００のポリエチレングリコールを用いて、常法により、脾臓細胞とマウス骨髄腫由来細胞株（Ｐ３Ｘ６３　Ａｇ８．６５３）とを融合させ、融合細胞を得た。

【００６５】融合細胞を、１５重量％のウシ胎児血清（以下、ＦＣＳ）を含むイシコフ培地で調製したヒポキサンチン／アミノプテリン／チミジン（ＨＡＴ）培地に浮遊させた後、９６ウェルプレート１枚にまいた（２００μｌ／ウェル）。この際、フィーダー細胞（培養開始時に成長因子を供給する細胞）は同じマウス個体の脾臓細胞を用いた。CO_2インキュベータ（CO_2濃度：５体積％、温度：３７℃、湿度：９５％）内で培養を開始した。以下の培養では、他に示さない限り、これと同じ条件で培養を行なった。

【００６６】（細胞選別およびクローニング）１週間後、融合細胞の培養上清を１００μｌ採取した後、融合細胞を含む残りの培養液を４枚の２４ウェルプレートに継代し、各ウェルに１ｍｌの１５重量％のＦＣＳを含むヒポキサンチン／チミジン（ＨＴ）培地を加えた。

【００６７】融合細胞を２４ウェルプレートに継代した４日後、細胞培養上清を１５０μｌ／ウェルずつ採取した。この培養上清と、培養開始後１週間目に採取した培養上清を用いて以下に示すＥＬＩＳＡ法により、ソブリヌスに対する結合能を測定した。

【００６８】固相として０．１ｍｇ／ｍＬ・ＢＳＡ－Ｐ

Ｂ Ｓ－Ａｚで５μｇ／ｍＬの濃度に調製したソブリヌスを、１００μｌ／ウェルずつ使用した。抗体液として、細胞培養上清を使用した。第２の抗体としてペルオキシダーゼ標識抗マウスＩｇＧ抗体を使用した。

【００６９】２回採取した培養上清のＥＬＩＳＡ法結果を合わせて、ソブリヌスに対して高い結合能を有する、増殖状態の良い２０ウェルを確認した。第１段階の選択として、これらのウェルの細胞を、すべて４枚の６ウェルプレートに継代し、各ウェルに４ｍｌの１５重量％のＦＣＳを含むＨＴ培地を加えた。

【００７０】第１段階の細胞選別の２日後、培養上清を採取し、以下に示すＥＬＩＳＡ法によりソブリヌスに対する結合能を測定した。

【００７１】固相としてＰＢＳ－Ａｚで10^8細胞／ｍＬの濃度に調製したソブリヌスを、１００μｌ／ウェルずつ使用した。抗体液として細胞培養上清を使用した。第二抗体として、ペルオキシダーゼ標識抗マウスＩｇＧ抗体を使用した。

【００７２】この結果、ソブリヌスに対して高い結合能を有したウェルを、１０ウェル選別した。これらのウェルの細胞を、それぞれ、中フラスコ（容量５０ｍｌ）に継代した。培地は１５重量％のＦＣＳを含むＨＴ培地を４５ｍｌずつ加えた。

【００７３】第２段階の選択を受けた細胞の継代３日後、培養上清を採取し、以下に示すＥＬＩＳＡ法によりソブリヌス、他のストレプトッコカス種（ストレプトコッカス・ミュータンス）に対する結合能を測定した。

【００７４】固相として、ＰＢＳ－Ａｚで10^8細胞／ｍＬの濃度に調製したミュータンスを、１００μｌ／ウェルずつ使用した。抗体液として細胞培養上清を使用した。第二抗体として、ペルオキシダーゼ標識抗マウスＩｇＧ抗体を使用した。細胞培養上清中に、ミュータンスに結合する抗体が存在するとウェル上に発色する。

【００７５】ソブリヌスでのみ結合能を示し、他のストレプトッコカス属細菌であるミュータンスに結合能を示さないウェルを、２ウェル選別した。

【００７６】上記２ウェルの細胞について、１５重量％のＦＣＳを含むＨＴ培地を用いて、１ウェルあたり２個の細胞が含まれる濃度に希釈（限界希釈）し、９６ウェルのマイクロプレート各２枚に分注した。フィーダーとして生後４週の雌のマウス（ＢＡＬＢ／Ｃ）の胸腺細胞を用いて初期増殖を促した。プレートのサイズを上げながら培養を進め、適時細胞培養上清について上記のＥＬＩＳＡ法によるスクリーニングを繰り返した。ソブリヌスに対して高い力価を示し、かつ良好な増殖を示している細胞株を最終的に選別し、２００ｍＬの培地中で5×10^5細胞／ｍＬの濃度に至るまで培養を進めた。最終的に、ソブリヌスに対して高い結合能を有し、かつ他のストレプトッコカス属細菌に対して交叉反応を起こさない株を２株選定した。

【００７７】ソブリヌスに対して高い結合能を示した中の1株を細胞株名：ＳＳ－１と命名し、工業技術院生命工学工業技術研究所に平成１２年６月３０日に国内寄託した（受託番号ＦＥＲＭ　ＢＰ７２０２号）。

【００７８】同様にソブリヌスに対して高い結合能を有したもう1株を細胞株名：ＳＳ－２と命名し、工業技術院生命工学工業技術研究所に平成１２年６月３０日に国内寄託した（受託番号ＦＥＲＭ　ＢＰ７２０３号）。

【００７９】ＳＳ－１株およびＳＳ－２株の産生する抗体を、それぞれ、ＳＳ１抗体およびＳＳ２抗体と称する。

【００８０】（細胞の保存）最終的に選別された細胞株は、遠心分離して上清を取り除き、１×１０^{7}細胞／ｍＬの濃度でＦＣＳ：ジメチルスルフォキシド＝９：１（体積比）の溶液１ｍＬに浮遊させ、－８０℃で予備凍結した後、液体窒素中に移して長期保存状態にした。

【００８１】（抗体の精製）選択した２株を、それぞれ、１５重量％ＦＣＳを含むイシコフ培地で大量培養し、その上清を遠心分離した。また、選択した２株を、それぞれ、雌のＢＡＬＢ／Ｃマウスの腹腔内に注射して増殖させ、腹水を蓄積させた。蓄積した腹水を採取した。各株の培養上清あるいは腹水を、プロテインＡ結合ゲル（プロテインＡセファロース４ＦＦ、ファルマシア製）を用いたアフィニティークロマトグラフィにかけ、以下の条件で各モノクローナル抗体（ＳＳ１抗体およびＳＳ２抗体）を精製した。

【００８２】プロテインA結合ゲルを充填したカラムを、結合緩衝液（１．５Ｍ　グリシン・３Ｍ　ＮａＣｌ、ｐＨ８．９）で平衡化した。培養上清あるいは腹水を、結合緩衝液で約３倍に希釈した後、平衡化したカラムにアプライした。カラムからの溶出液を２８０ｎｍでモニターしながら、不純物の溶出が終了するまで、カラムを結合緩衝液で洗浄した。洗浄後、溶出緩衝液（１００ｍＭクエン酸、ｐＨ４）をカラムにアプライ（線流速：約２０ｃｍ／時間）し、ＩｇＧ含有溶出液を回収した。回収したＩｇＧ含有溶出液について、吸光光度計で２８０ｎｍの吸光度を測定し、測定された吸光度を吸光係数で換算することにより、抗体の濃度を決定した。

【００８３】ＳＤＳポリアクリルアミドゲル電気泳動による標準タンパク質との比較から、これらのモノクローナル抗体（ＳＳ１およびＳＳ２）の精製分画は、いずれも分子量約５０，０００のH鎖と約２５，０００のＬ鎖からなるＩｇＧであることを確認した。なお、電気泳動上で、不純物の混入は検出限界以下であった。

【００８４】（抗体の評価）上記のアフィニティークロマトグラフィにより精製した２種類のモノクローナル抗体について、ソブリヌスの希釈系列を用いて、上記第２段階の選択におけるインヒビションＥＬＩＳＡ法と同一条件で抗体評価を行った。

【００８５】図１は、ＳＳ１抗体について、図２は、同様にＳＳ２抗体について、それぞれ（ａ）ソブリヌス（白丸）、（ｂ）ミュータンス（黒丸）に対する結合能を測定した結果を示すグラフである。

【００８６】図１に示すように、ＳＳ１抗体では、×１～×１／１０希釈液でソブリヌスに対する結合が観察されたのに対し、ミュータンスに対してはいずれの希釈液についても結合は観察されず、ＳＳ１抗体によって特異的にソブリヌスを検出し得ることが示された。

【００８７】同様に、図２に示すように、ＳＳ２抗体においても、×１～×１／１００希釈液でソブリヌスに対する結合が観察されたのに対し、ミュータンスに対してはいずれの希釈液についても結合は観察されず、ＳＳ２抗体によって特異的にソブリヌスを検出し得ることが示された。

【００８８】（サンドイッチ反応）ＥＬＩＳＡ法においてＳＳ１抗体をプレートにコートし、ソブリヌスを結合させ、酵素ラベルしたＳＳ２抗体を反応させた後に余分な抗体を除去し、発色基質を添加してインキュベートしたところ、充分な発色が得られた。つまり、ＳＳ１抗体と、ＳＳ２抗体またはポリクローナル抗体との組合せは、免疫クロマトグラフィーなどのサンドイッチ反応を利用した検査方法に有用であることが確認できた。

【００８９】（免疫クロマトグラフィーにおける検出感度）常法に従ってＳＳ１抗体を濾紙上に固定化し、金コロイド標識したＳＳ２抗体を移動相に置いて、免疫クロマトグラフィー装置を作製した。種々の濃度でソブリヌスを含むサンプルをアプライしたところ、この免疫クロマトグラフィー装置の感度は、約１０^{6}細胞／ｍｌであった。

【００９０】一般に、健常人の口腔内中のソブリヌスレベルは、約１０^{4}～１０^{7}細胞／ｍｌであることが知られている。従って、本発明の抗体を用いて作製された免疫クロマトグラフィー装置は、口腔内中のソブリヌスを検出することが可能である。

【００９１】

【発明の効果】本発明のモノクローナル抗体産生細胞株の作製方法によれば、細胞株のクローニングにおいて、融合細胞から産生される抗体のソブリヌス、他のストレプトッコカス種に対する結合能を検定し、目的の細胞を選別する。そのため、細胞融合の後、初期に存在する多くの細胞から、効率よく有用な細胞を選択し得る。そして、ソブリヌスに対する高い親和性を達成しながら、特異性の高い抗体を産生する細胞株を作製し得る。

【００９２】免疫測定法が酵素免疫測定法（ＥＬＩＳＡ法）である場合、抗体の結合能を簡便に感度よく検定し得る。

【００９３】哺乳動物の骨髄腫由来の細胞株が、マウス骨髄腫由来Ｐ３Ｘ６３　Ａｇ８．６５３である場合、抗体の断片を産生せず、さらに得られる融合細胞の増殖能力が特に優れているため、短時間に多くの細胞を検定し

得る。

【００９４】免疫する哺乳動物がマウスまたはラットである場合、動物の取り扱い、免疫感作の点で都合がよい。免疫する哺乳動物がＢＡＬＢ／Ｃ系統マウスである場合、ソブリヌスとの親和性が極めて高いモノクローナル抗体を得ることが可能となる。

【００９５】本発明のモノクローナル抗体産生細胞株によれば、それを培養することによりソブリヌスに対する高い親和性を有し特異性の高い抗ソブリヌスモノクローナル抗体を半永久的に提供し得る。

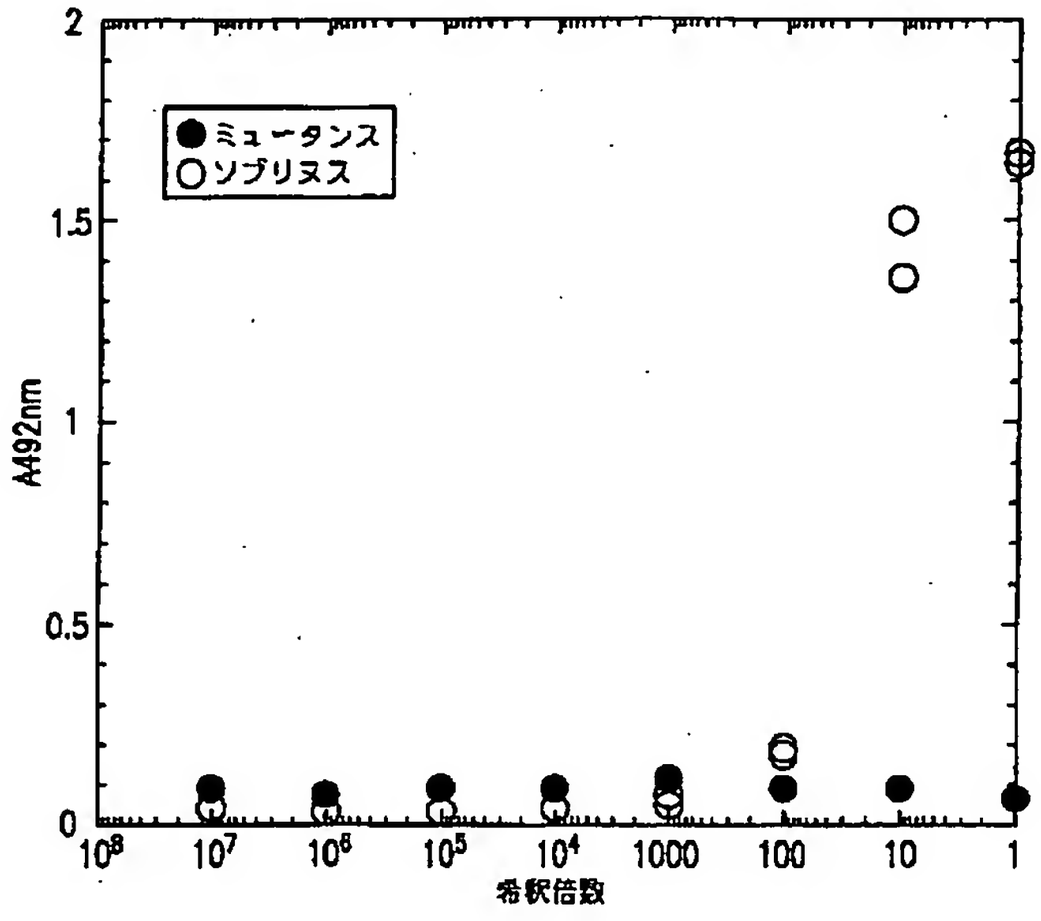
【００９６】本発明のモノクローナル抗体である、ＳＳ１抗体とＳＳ２抗体とを組み合わせて、あるいは、本発明のモノクローナル抗体と、抗ソブリヌスポリクローナル抗体とを組み合わせてサンドイッチ法に使用すれば、高特異的なソブリヌス検出キットを提供し得る。

【図面の簡単な説明】

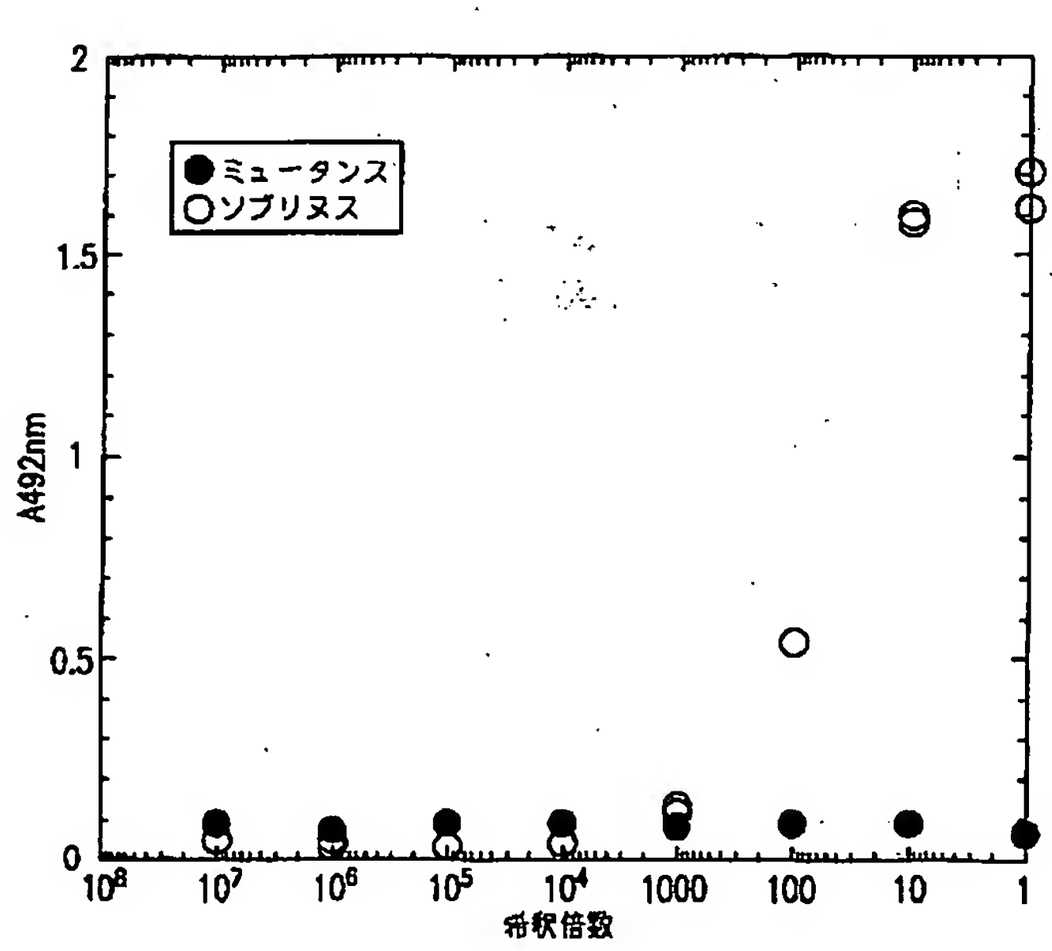
【図１】ＳＳ１抗体を用いた、ＥＬＩＳＡ法の結果を示すグラフである。グラフのデータは、本発明の抗ソブリヌスモノクローナル抗体（ＳＳ１抗体）のソブリヌス及びミュータンスに対する結合能を示す。

【図２】ＳＳ２抗体を用いた、ＥＬＩＳＡ法の結果を示すグラフである。グラフのデータは、本発明の抗ソブリヌスモノクローナル抗体（ＳＳ２抗体）のソブリヌス及びミュータンスに対する結合能を示す。

【図１】



【図２】



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